Strategic Nutrient Enhancement of Mustard Oil Seed Cake by Briquetting and Koji Fermentation for Ruminants Feed

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Research Article

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Abstract

Purpose

Edible oil industries are shifting to the increased production of cold pressed oils in order to preserve some of the vital nutrients the oil. Consequently, the seed cake residue would lack significant nutrients that are otherwise retained in the oils, thus making the quality of residue inferior when applying as cattle feed.

Methods

In this study, mustard oil seed cake (MOSC) was employed as the substrate and the nutritive and feed quality were enhanced using Koji strains viz., *Aspergillus oryzae* and *Aspergillus niger*, separately and suitable process parameters such as Solid: Liquid, pH, incubation time and inoculum quantity were optimized for the maximum nutritive enhancement of cold pressed MOSC. Changes in physico-chemical properties were analyzed by SEM, EDS, FTIR along with feed functional properties to analyze the quality. Briquetting of MOSC was employed for enhanced microbial encroachment.

Result

Free amino acid (FAA) and reducing sugar (RS) were chosen as critical indicators of enrichment. *A. oryzae* resulted in 20.74 fold and 19.07 fold increase in FAA and RS respectively, whereas, *A. niger* resulted 13.24 fold and 3.04 fold increase. Critical parameters such as solid:liquid, pH, time and inoculum volume were selected. Briquetting resulted in efficient mycelia coverage as evident from SEM images and EDS analysis indicated enhancement in essential elements in the MOSC. Functional properties after fermentation indicated an effective transformation of MOSC.

Conclusion

Utilizing these seed cakes as cattle feed not only provide the required nutrition, but also helps in the efficient utilization of the residual waste oilseed cakes.

Novelty

Recently, to enhance the nutritive factors, edible oil industries are switching from hot pressed to cold pressed oils to retain heat labile compounds like vitamins and carotenoids in the oils, on the other hand, it results in lesser nutritional content in the cake residue. Therefore an inferior quality and presence of anti-nutritional factor restricts the use of this cold pressed mustard oil seedcake as feed. Supplementing Koji fermented oilseed briquettes as feed obtained through our research may alter the rate of nutrient digestion in the lactating cows that consequently beneficial to the dairy industries. Utilizing these seed cakes as cattle feed not only provide the required nutrition, but also helps in the efficient utilization of the residual waste oilseed cakes.

1. Introduction

Dairy industries play an extremely important part in the Indian economic development, contributing to about 33% of the gross income. Milk and its products provide an immense value in the agricultural and food sector in India. About 4% of India's GDP is contributed by the livestock sector, and the majority of it shared by the dairy industries. This brings the need for the production and maintenance of healthy dairy cattle, and developing an effective feed that can provide all the vital nutrients. Throughout centuries animal-source food has played a vital role in the human development. Livestock development also contributes to the economic development of the lower income countries. This gives the priority and the need for developing technologies for maximizing the productivity of these animals. Apart from producing food and generating income, livestock also plays a role in providing manure, serves as financial instruments and enhances the social status [1].

Dairy farming is especially important for the marginal farmers. Studies have shown that 60-65% of the income of the small-scale farmers comes from the dairy farms. The dairy industry also plays a major role in improving the socio-economic development by positively influencing the lives of the people who are involved in this business [2]. The need of efficient animal feed sources brought the attention towards pressed oilseed cakes as an alternative to be used as a feed, which is a by-product produced after the oil has been extracted. The consumption of edible oils in India has risen dramatically, making it the second largest consumer, the largest being China [3]. Many different types of oilseed crops are produced in the wide range of climatic conditions of India. India has the fourth largest edible oil economy and contributes to about ten percent of the world oilseed production. Groundnut, soybean and mustard rapeseed are the three major oilseeds produced in India, accounting for more than eighty eight percent of total oilseed output. Mustard-rapeseed is the second most important oilseed crop next to soya bean, majorly grown in Rajasthan, Haryana, Madhya Pradesh, West Bengal, Uttar Pradesh and Gujarat, where the estimated production in 7.1 million tonnes, accounting for 93% of the total production in the country [4].

More recently, cold press extraction is used for the extraction of the oils as it requires less energy and is more environment-friendly [5]. High quality oils can be extracted when performed under less harsh, low temperature using this cold press method. Thus, the oil obtained contains more nutritious, heat labile components like carotenoids that were otherwise destroyed when extracted by hot press method. An increase in the awareness in the population about the health benefits of these cold pressed oils would possibly increase the production of the cold pressed seed cakes to a large amount in the near future. Therefore, utilizing these cold pressed oilseed cakes as a feed would serve as an efficient way for utilization of the agricultural waste.

Koji mould is capable of synthesizing various enzymes (intracellular as well as extracellular) the can hydrolyse various carbohydrates (amylose, maltose, cellulase, xylose, amyloglucosidase) proteins and to some limited extent lipids as well [6,7]. The products (sugars, amino acids and lower molecular weight peptides) are needed for the soya sauce production, in the fermentation step. However, the moulds need to be inspected carefully and check for the presence of aflatoxins and other mycotoxins like Kojic acid under certain conditions [8]. In line with the aforementioned advantages of Koji fermentation, there are lot

of studies that are reported on its application to agro-byproducts other than conventional raw materials such as walnut meal [9], rice dreg protein [10] and millet powder [11].

Mustard oilseed cake (MOSC) has a good amount of protein and a good balance of sulphur-rich amino acids, which often serves as a limiting factor for cattle growth and development. MOSC, even though is protein rich, contains rumen undegradable protein (RUP). Cold-pressed MOSC contains 460g per kg RUP [12]. However, the RUP content differs depending upon various conditions like maturity during the harvest, techniques use for sample preparation and the conditions during the extraction procedures. MOSC could serve as an alternative feed source, however not much information on the percentage of feed to be given is available. It contains high amount of bioactive compounds like phenolics, which may be present in free or esterified form. It may also be conjugated with insoluble compounds. However, the bitter taste contributed by the tannins makes it less palatable to the ruminants [3]. Therefore, such anti-nutritional factors (ANFs) need to be alleviated and increase easy assimilable sugar and essential amino acids in MOSC to serve as a potent cattle feed.

In this study, solid state fermentation (SSF) was employed to observe the effect of Koji strains such as *Aspergillus niger* and *Aspergillus oryzae*, separately, on improving the levels of free amino acids and reducing sugar content of the cold pressed MOSC and process parameters like time, pH, inoculum volume and moisture content were screened and optimized for maximum nutrient enrichment. Further, changes in functional properties, levels of anti-nutritional factors, morphology and functional groups were also investigated, before and after fermentation thereby taking a step forward to establish it as a feed.

2. Materials And Methods

2.1 Microorganisms and Raw material

Aspergillus oryzae MTCC 3107 and *Aspergillus niger* were procured from the Institute of Microbial Technology (IMTECH) culture collection bank, Chandigarh, India. These strains were subcultured in Potato Dextrose Agar (PDA) media for its subsequent use as an inoculum in Koji fermentation process. Both hot and cold pressed MOSC were obtained from local oil extraction units. The substrates were ground in a ball mill to reduce and sieved to obtain the particle size of 0.5 mm.

2.2 SSF of MOSC with Koji technique

SSF was performed in Petri plates with cold- and hot-pressed MOSC as the substrates were inoculated with the fungus. Solid state fermentation (SSF) is defined as the fermentation involving solids (substrates) in absence or near absence of free water or moisture [13]. Media components such as KH_2PO_4 (0.02 g), $MgCl_2$ (0.01 g), NaCl (0.01 g) were taken in 10 mL of water to utilize as the basal media for fermentation. The prepared media was mixed to the MOSC in minimum amount to make it moist. The solid substrates should not have any free-flowing liquid. After autoclaving at 121°C, about five pieces of ~1 cm^2 agar cubes of Koji strains (spore count for *A.niger* and *A.oryzae* were found to be 1.714×10^6 spores/mL and 9.86×10^5 spores/mL respectively) were cut and dispersed into the plates containing the

substrates. The plates were then left for incubation at 35 ºC and growth of the fungus was monitored. After five days of growth the fungal mycelia had effectively penetrated the inside of the solid substrate voids.

2.3 Briquetting process

Briquetting process was done to increase the surface area of the oilseed cake for uniform fungal growth and subsequent mycelial encroachment. About 5 g of MOSC was taken in a 100 mL glass beaker and minimal media was added to it. After that, oilseed cakes were inoculated with both the fungal species of *Aspergillus oryzae* and *Aspergillus niger* and briquettes were made using a hand-held extruder.

2.4 Biochemical characterization of MOSC

The characterization of nutrients present in the MOSC was performed using estimation of Carbohydrate content [14], reducing sugar content [15], protein content [16] and free amino acid content [17].

2.4.1 Extraction and estimation of free amino acid content using Ninhydrin assay

About 500mg of the MOSC was ground to a thick paste using 80% (v/v) ethanol. The supernatant obtained after centrifuging was tested for the presence of free amino acids using standard Ninhydrin assay which involves addition of 1mL of 8% Ninhydrin solution in acetone to the diluted sample. This is further followed by heating in a boiling water-bath along with some marble chips for preventing evaporation related losses for about 15 min, after which 1 mL of 50% (v/v) ethanol was added to the heated sample in order to halt the ongoing reaction thereby preventing any change in the optical density (OD) taken. The OD was then taken at 570 nm while keeping water as the blank.

2.5 Selection of process parameters for optimal SSF

The conventional one variable at a time (OVAT) approach was used to select the significant parameters and the initial test range of the four variables i.e. Solid:Liquid, time (days), inoculum volume (mL) and pH for fermenting cold pressed MOSC inoculated with *Aspergillus oryzae* and *Aspergillus niger*. The effect of these parameters on the nutrient enrichment was checked varying one parameter at a time and keeping the other parameters and process conditions constant. Reducing sugar and amino acid content were kept as an indicator for nutrient enrichment. All the experiments were performed in triplicates and the mean and standard deviation were calculated through Microsoft Excel 2013.

2.6 Analysis of functional properties

All the functional properties were calculated according to the methods given by Sadh et al. [18].

2.6.1 Bulk density

MOSC was filled in a 10mL cylinder and tapped gently. The powder was weighed and bulk density was calculated as mass of sample per unit volume of sample.

2.6.2 Water binding capacity

About 1g of MOSC was mixed with 15 mL of deionized water and was held for 30 minutes. Centrifugation was done at 3000 rpm for 10 minutes. Water binding capacity was expressed as gram of water retained per gram of sample.

2.6.3 Oil binding capacity

About 1g of MOSC was mixed with 15 mL of coconut oil and was held for 30 minutes. Centrifugation was done at 3000 rpm for 10 minutes. Oil binding capacity was expressed as gram of oil retained per gram of sample.

2.6.4 Emulsifying Capacity (EC)

About 1% sample was sonicated with 25mL of coconut oil with 5s pulse rate for 15 minutes. Emulsions were centrifuged at 11000 rpm for 5 minutes. The formula for calculating the EC is given in Eq.1.

$$EC\% = \left(\frac{Height of emulsified layer}{Height of total content in the tube}\right) * 100 \quad (1)$$

2.6.5 Emulsifying Stability (ES)

Emulsion was heated at 80 ºC for 30 minutes and centrifugation was done at 11000 rpm for minutes. The formula for calculating ES is given in Eq.2.

$$ES\% = \left(\frac{Height\ of\ emulsified\ layer\ after\ heating}{Height of emulsified\ layer\ before\ heating}\right) * 100 \quad (2)$$

2.6.6 Foaming Activity (FA)

About 3% (w/v) solution of MOSC sample was mixed with deionized water for 45 minutes on magnetic stirrer followed by blending. Volume was measured before and after stirring. The formula for calculating the FA is given in Eq.3.

$$FA\% = \left(\frac{Final\ volume - Initial\ volume}{Initial\ volume}\right) * 100 \quad (3)$$

All the experiments were carried out in triplicates (n=3) and the values plotted in the graphs/figures is average± SD.

2.7 Scanning Electron Microscopic (SEM) Analysis

SEM analysis was performed to provide high resolution imaging of unfermented as well as fermented briquettes of cold pressed MOSC and to investigate the morphology of briquettes after inoculation with *A.oryzae* and *A.niger*. The briquettes were ground well before being analyzed. The samples were studied

using FEI Quanta 200 FEG – High Resolution Scanning Electron Microscope. SEM-Energy Dispersive X-Ray Spectroscopy (EDS) analysis was also performed to obtain the elemental composition of the fermented and unfermented MOSC.

2.8 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis was done to analyze the changes in the organic and polymeric functional groups present in the fermented and unfermented MOSC. The infrared spectra of the samples were analyzed using Fourier Transform Infrared Spectrometer (M/s. IRTRACER 100). The sample was loaded on the sample holder and it was scanned from 4000 to 500 cm^{-1} wave range.

2.9 Estimation of ANF component in terms of tannin content

The extraction and estimation of tannins was performed before and after SSF for both *A.niger* and *A.oryzae*. For extracting tannins, about 50mg of the powdered samples were boiled with 7.5 mL of distilled water for about 30 minutes followed by centrifuging for about 10 minutes at 3000 rpm. The supernatant obtained was analyzed using the standard Folin-Denis spectrophotometric method which involves addition of 0.5 mL of Folin-Denis reagent and 1mL of filtered sodium carbonate solution to 1mL of sample in a covered glass test tube. This was further diluted to make the total volume to 10 mL. The test tube was then shaken properly and the absorbance was measured at 700 nm.

3. Results And Discussion

3.1 Characterization of MOSC

MOSC are typically yellow to brown in color. They have a pleasant smell and characteristic odor as reported in earlier studies [19]. The intensity of this odor varies in different type of mustard. It was observed that the carbohydrate, reducing sugars and free amino acids concentration was lower in case of cold pressed MOSC than in hot pressed MOSC (Table 1). However, the protein concentration was found to be higher in case of cold pressed MOSC (13.08 mg/g of biomass). This can be directly related to the difference in the production processes and health benefits of cold pressed over hot pressed oils. In cold pressed oils, the heat-labile components like proteins and vitamins are not degraded [20]. In general, ruminants need amino acids for protein synthesis required for proper metabolism, growth, lactation and reproduction. Mostly ruminants depend on microbial proteins synthesized in rumens and from dietary feed supplementation that are un-degraded in the rumen. In spite of these routes, dependence of production of microbial protein in rumen solely is insufficient to supply required quantity of amino acids for optimal metabolism [21]. Therefore, the cattle feed should adequately constituted with these free amino acids thereby the limiting amino acids shall be supplied through consumption patterns. The objective of this study is to enrich such free amino acids through adoption of the Koji fermentation.

3.2Verification of Koji fermentation on the hot pressed and cold pressed MOSC

From Fig. 1(a), it was observed that the carbohydrate concentration decreased after fermentation of the MOSC (hot pressed) with both the *Aspergillus* sp. A subsequent increase in the concentration of reducing sugars was observed after fermentation. *A.oryzae* showed a significant increase in reducing sugar concentration (11.72 mg/g of MOSC) as compared to *A.niger* (2.5 mg/g of MOSC) after 5 days fermentation of the oilseed cake (Fig. 1 (c)). This can be attributed to the fact that *A.oryzae* MTCC 3107 is an efficient producer of amylase [22] and therefore breakdown the starch into simpler sugars, in addition to breaking down lignin and cellulose. *A.niger* is a well-known ligninase and cellulase producer and thereby degrades the lignin to make the cellulose accessible to the enzymes [23]. From Fig. 1 (b), it was observed that the protein concentration decreased after fermentation of the MOSC (hot pressed) with both *Aspergillus* sp.

A subsequent increase in the concentration of free amino acids was observed (Fig. 1 (d)) after fermentation. Being an efficient protease producer, *A.oryzae* showed a significant increase in free amino acids concentration (2.88 mg/g of MOSC) as compared to *A.niger* (1.634 mg/g of MOSC) after fermentation of the oilseed cake [22]. Briquetting of the cold pressed MOSC was done using hand-held extruder as shown in the Appendix A as (Fig. A1). This resulted in uniform growth of the mycelia around the briquetted oilseed cake as well as inside the briquettes when compared to the powdered form of the substrate. Earlier, non-uniform growth of the mycelia was observed as the there was no solid support for fungal attachment and growth. Thus, briquetting of the oilseed cake resulted in uniform growth of the mycelium around the cold pressed MOSC, ease in feeding, packaging and transportation of the oilseed cake in large scale field application. The dimension of each briquette was measured and the measurements were found out to be as follows: diameter =1.3 cm; height = 0.9 cm; surface area = 6.34 cm^2.

A significant increase in the concentration of reducing sugar and free amino acids deciphers the breakdown of polymers into monomers or simpler units by the action of enzymes released by *Aspergillus* sp., [24]. Even other authors who employed Koji fermentation in agro-byproducts have used increase in sugar and amino acids as a critical indictor of the process efficiency [9,10]. Hence, free amino acids and reducing sugar were chosen as the dependent factors for the selection of process parameters using two different *Aspergillus* sp., as these two plays a vital role in cattle feed quality of MOSC.

3.3 Selection of process parameters for solid state fermentation

Four process parameters (Solid to liquid ratio; incubation time; pH; inoculum volume) that affect fungal growth and fermentation were chosen and selection of the optimum values for each of these process parameters were done using OVAT approach. The effects of different parameters have been schematically represented in Fig. 2 (a-h).

3.3.1 Effect of Solid: Liquid

Solid: liquid which indicates the moisture content plays a critical role in the growth of any microorganism. Alteration in the moisture content can lead to changes in the end-product. Higher moisture content would

lead to increased humidity whereas lower moisture content would result in dry conditions inadequate for fungal growth [25]. Fig. 2 (a) and (b) show the effect of varying moisture content on SSF of cold pressed MOSC using *A.oryzae* and *A.niger*, respectively. For *A.oryzae*, the maximum production of free amino acids (5.759 mg/g of MOSC) and reducing sugar (4.583 mg/g of MOSC) were observed with 2.5:1 solid: liquid which were found to be 8.22 and 2.29 fold increase as compared to control respectively. Similarly for *A.niger*, the maximum production of reducing sugar (6.6 mg/g of MOSC) and free amino acids (1.928 mg/g of MOSC) were observed with 2.5:1 solid: liquid with 3.3 and 2.82 fold higher than the unfermented MOSC. Thus, 2.5:1 solid: liquid ratio was chosen as the optimum for both the organisms. The comparison also elucidated the potential of *A.oryzae* and *A.niger* efficient in proteolytic and saccharolytic properties respectively.

3.3.2 Effect of pH

Understanding of pH is important as it affects the growth and sporulation of the microorganism. From Figs. 2 (c) and (d), it can be clearly deciphered that there is a gradual increase in free amino acid from initial pH of 6 to 7 (Fig. 2 (c)) and there was a maximum yield at 7.5 and 8 for *A.oryzae* and *A.niger* respectively which indicates that both the strains prefer the neutral to slightly alkaline range for followed which there was steep decline. Whereas, for *A.oryzae* (Fig. 2 (d)), the maximum production of reducing sugar (5.44 mg/g of MOSC) was observed at pH 7. Similarly for *A.niger*, the maximum production of reducing sugar (5.45 mg/g of MOSC) wasobserved at pH 8. Thus, pH 7 for *A.oryzae* and pH 8 for *A.niger* were chosen as the optimum pH as the enzymatic (protease, amylase, cellulase, etc.) activity was found to be highest at these pH as reported by Abubakar et al. [26].

3.3.3 Effect of incubation time

Incubation time plays an important role in sporulation and enzymatic activity of fungi. Ideally, fungal growth and enzyme production is optimum between 2-5 days [27], which forms the basis for choosing the range in the present investigation. Figs. 2 (e) and (f) show the effect of incubation time on SSF of cold pressed MOSC using *A.niger* and *A.oryzae* respectively. For *A.oryzae*, the maximum production of free amino acids (13.35 mg/g of MOSC) and reducing sugar (5.416 mg/g of MOSC) were observed on the 4^{th} day of incubation which is 2.72 and 19.02 fold respectively higher as compared to 0^{th} day. Similarly for *A.niger*, the maximum production of reducing sugar (6.042 mg/g of MOSC, 2.72 fold increase) and free amino acids (9.272 mg/g of MOSC, 13.11 fold increase from 0^{th} day) were also observed on the 4^{th} day. The fold increase in *A.niger* was however found to be less as compared to *A.oryzae*. Thus, the 4^{th} day of incubation was chosen as the optimum time of incubation for both the organisms. Longer incubation time at elevated temperature might lead to inter-reaction between the reducing sugar and amino acid in an aqueous condition like Milliard reaction [9] that might be the plausible reason for decrease in the sugar and free amino acid when incubated for 5 days.

3.3.4 Effect of inoculum volume

Inoculum volume or the spore count, plays a very important role in the process of fermentation. Higher inoculum volume can lead to scarcity of oxygen and nutrients in the medium whereas lower inoculum volume leads to lesser biomass formation. Fig. 2 (g) and (h) exhibit the effect of varying inoculum volume on SSF of cold pressed MOSC using *A.oryzae* and *A.niger*, respectively. For *A.oryzae*, the maximum production of free amino acids (14.52 mg/g of MOSC) and reducing sugar (4.652 mg/g of MOSC) were observed with 3 mL of inoculum which amounts to 20.74 and 2.32 fold higher as compared to control. For *A.niger*, the maximum production of reducing sugar (8.159 mg/g of MOSC, 4.07 fold increase) and free amino acids (4.336 mg/g of MOSC, 6.19 fold increase) was observed with 2.5 mL of inoculum. Thus, 3mL for *A.oryzae* and 2.5mL for *A.niger* were chosen as the optimum inoculum volume. From the overall effect of parameters, it can be clearly observed that the fold increase in nutrient content found to elevate with changes in the process parameters. Further, statistical optimization is required from the range chosen from this study to arrive at the exact combination of effective parameters towards maximum nutrient enhancement.

3.4 Functional properties of fermented cold pressed MOSC

Since MOSC is relatively cheaper than most other oil seed cakes like peanut and soyabean oil seed cakes and produces lesser aflatoxins than groundnut oil seed cake, the functional properties of MOSC were tested to check if fermentation can improve the functional properties to be more easily digestible by the cattle. The functional properties of control, *A. niger* and *A.oryzae* fermented MOSC are tabulated in Table 2.

3.4.1 Bulk density of fermented MOSC

The bulk density of powder sample influence the texture, and the amount and strength of packaging material required for its distribution [28]. It has been recommended to reduce the bulk density of the feed. After subjecting to SSF, there was a reduction in bulk density to about 27.59% and 22.41% as compared to the unfermented (control) with *A. niger* and *A. oryzae* respectively (Table 2). The advantage of decreased bulk density of the fermented sample is in better packaging as well as low bulk food material [29].

3.4.2 Water and oil binding capacity

Water binding capacity significantly affects the inter-meal gap in cattle whereas the oil binding capacity plays an important role in flavor retention and texture of the feed. Both of these properties are in inverse correlation where a feed is expected to be in decreased water binding and increased oil binding ability which is in well correlation with the fermented MOSC. From Table 2, the hierarchy amongst the analyzed samples for water binding capacity was found to be Control>*A.niger*>*A.oryzae* whereas for oil binding it was Control<*A.niger*<*A.oryzae*. Therefore, *A.oryzae* found in superior quality as compared to *A.niger* fermented in terms of feed digestibility. Fermentation causes unfolding and modification of macromolecules of the products, The unfolding exposes the hydrophilic domains of the amino acid residues of proteins and other macromolecules which have a higher affinity for the aqueous medium. In

this higher value of water binding capacity indicates that fermentation process resulted in an increased number of exposed hydrophilic interactions as compared to oil binding capacity and unfermented sample. Therefore, the fermented product is easily digested in comparison to unfermented product. These factors significantly influence the composition, physical structure, porosity, and particle size of the dried cake powder.

3.4.3 Foaming activity

Foam formation and stability are dependent on properties like pH, viscosity, surface tension and the processing methods employed which is directly related to the presence of surface soluble proteins [18].The foaming property is decreased from 11.26% (control) to 5% and6.70% with *A. oryzae* and *A.niger* respectively, because of the protein content also decrease after the fermentation of sample as indicated from the SSF potential studies.

3.4.4 Emulsifying capacity and stability

Emulsifying capacity and stability were found to be increased in the fermented MOSC, thus indicating improved digestibility of fats. Emulsifying capacity signifies the maximum quantity of oil that can be emulsified through dispersion, whereas emulsion stability elucidates the ability of an emulsion with a certain composition to remain unchanged. The fungal proteolytic activity might have exposed hydrophobic groups which resulted in the change of hydrophilic-lipophilic balance (HLB) that eventually favored emulsification [30]. High HLB surfactant are generally water-soluble whereas low HLB surfactant is oil soluble, enzymatic hydrolysis during fermentation process generally results in improving emulsifying activity by producing lower molecular weight peptide that easily migrates into the oil-water interface.

3.4.5 Morphology characterization of fermented MOSC by SEM

The unfermented and fermented briquettes were analyzed by SEM to understand the fungal coverage morphology better and observe the growth of the mycelium inside the briquetted oilseed cake. Figs. 3 (a-g) depict the SEM images of the fermented briquettes at different magnifications and control (unfermented briquette). The dense growth of mycelium of *A.oryzae* was observed that percolated even inside the briquettes. The presence of distinct spores of *A.niger* was observed even inside the briquettes. While performing briquetting process the MOSC moistened with media and mixed well with the spores and extrudated to form briquettes. This ensures uniform microbial distribution in the solid substrate where inoculum dispersion is considered as one of the bottlenecks of SSF while using powdered biomass.

3.5 Elemental analysis by SEM-EDS

SEM-EDS analysis of the unfermented and fermented briquettes was performed to understand the change in the elemental composition of the briquetted oilseed cake. Fig. 4 (a-c) represents the EDS spectra for the unfermented cold pressed MOSC. The composition of elements present in different MOSC

has been tabulated in Appendix (Table A1). From the obtained results, it has been observed that C-51.61 wt%, O-45.27 wt%, K-1.19 wt% and Ca-Mo traces were found to be the major elements in the unfermented cold pressed MOSC. Whereas with the briquettes fermented with *A.oryzae*, C-57.43 wt%, O-37.21 wt%, S-0.96 wt% and traces of Ni-P-Al were found to be the major elements of the fermented oilseed cake. In case of *A.niger,* C-42.54 wt%, O-47.73 wt%, K-1.57 wt%, Ca-1.81 wt%, Mg- 1.66 wt%, P- 1.49 wt% and traces of S-Al-Na were found to be the major elements of the fermented oilseed cake. In cattle diets, calcium, phosphorus and sodium are the major limiting elements. From Fig. 4 (a, b and c), it has been observed that calcium, phosphorus, sulfur, sodium, nickel and aluminum are present in the cold pressed MOSC after fermentation with *Aspergillus* sp. Calcium and phosphorus play a very important role in the development of the skeletal system and lactation in cattle. Deficiency in either or both causes a decrease in ability to gain weight and formation of weak bones. Cattles provided with a diet richer in calcium tends to provide superior quality milk than the ones lacking it. Sulfur acts as a precursor for the formation of cysteine and methionine, which in-turn promote lactation in cows. Sodium plays a vital role in pH regulation, water absorption and proper functioning of the nervous and muscular systems. Sodium deficiency in cattle may lead to decrease in weight gain and appetite [31]. Nickel in cattle feed supplement improves feed efficiency and ruminal urease activity in ruminants [32]. Aluminium is known to alter the metabolism of other minerals as well as reduction of toxicosis in ruminants [33]. Since MOSC fermented feed supplement has all these essential macronutrients, the above mentioned problems due to elemental deficiencies can be alleviated by using SSF approach.

3.6 Functional group analysis by FTIR

The unfermented and fermented cold pressed MOSC were analyzed by FTIR to observe the changes in the chemical structure and functional groups, before and after fermentation. From the FTIR spectra as shown in Fig 5 (a-c), there were clear differences in control and fermented samples. FTIR analysis shows some prominent features, indicating some significant conversions during the process of fermentation. Appearance of peak at 1220-1250 cm^{-1} shows formation of ether after the process of fermentation. A similar observation was also noted by Shi et al. [34] which state an increase in the concentration of the ether extract with increase in incubation time. Change in the appearance of the peak at 2890-2925 cm^{-1} from sharp, strong to broad shows that there has been a conversion from methylene to methine group after fermentation. Disappearance of the peak at 1745 cm^{-1} indicates that there is a possibility of utilization or conversion of the esters by the micro-organisms. Appearance of the peak at 500-550 cm^{-1} shows the formation of chloroalkanes.

This is further supported by the study conducted by Shi et al., (2015), where an increase in the concentrations of trichloro-acetic acid soluble protein nitrogen (TCA-SP) was observed after fermentation. It is assumed the TCA-SP consists of small peptides and free amino acids. This is further supported by our results, which shows an increase in amino acid concentration with fermentation. The ester-carbonyl group was seen after fermentation [35]. External aquaphobes are related with the content of alpha-helix and in case of fermentation, improved oil binding capacity

significantly revealed the balance of hydrophilic and hydrophobic domain, as can be inferred from Aryee et al. [30].

3.7 Analysis of ANFs (Tannins) in Cold Pressed MOSC

Anti-nutritional factors (ANFs) are compounds that are produced in human and animal feed by normal metabolic processes that interfere with other nutrients uptake. This leads to a decreased metabolic performance in animals. The primary ANFs found in MOSC include tannins, phytic acid, glucosinates, saponin, etc [36]. Tannin content in control (unfermented seed cake) was found to be 5.10 mg/g of biomass. From the results as given in Fig.6, it has been observed that there has been a significant decrease in the concentration of tannins which is almost 60% reduction by 4^{th} day of incubation with *A.niger*, thus indicating that it is an efficient tannase producer, an enzyme that breaks down tannins. This is supported by the results shown by Knudson [37] which suggested that while *A.niger* is utilizing the organic compounds, it produces tannase and degrades tannic acid. This may also lead to a consequent accumulation of gallic acid.

However, *A.oryzae* was found to be an inefficient degrader of tannins. About 13.7% increase in the concentration of tannins was observed after the fourth day of fermentation. This is supported by the data provided by Sharath et al. [38] where it was suggested that the increase in the levels of tannins may be due to increase in free phenolic compounds after the fermentation process.

Even though both the strains were found to be efficient candidates for improving the property of MOSC, there are certain studies like amino acid profiling and toxicity analysis are to be done before employing it for the cattles. The research is being directed towards it for complete applicability.

4. Conclusion

This study has screened the parameters for maximum benefit and enrichment of the nutrient level in unutilized inferior MOSC from cold press processing units. The optimization of these process parameters through statistical techniques is underway. Through the proof of this study, it can be inferred that adoption of Koji fermentation in MOSC could serve as an alternate way to utilize the oilseed cake, which is an agricultural waste, thus beneficial for the industrialists. Farmers and animal breeders may also be benefitted due to the low cost and easy availability of the seed cakes, as well as providing sufficient nutrition to the cattle. Thus, our results suggest that SSF using Koji strains can serve as an effective way to enhance the nutrition and adapting this in large scale could have a positive impact on the agrarian society as well as industries. Similar strategies can be adopted for other industrial wastes such as cold pressed sunflower oilseed cakes, groundnut oil seed cakes, etc. as well as mixed cultures for further nutrient enhancement.

Declarations

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Conflicts of interest/Competing interests: This article is an original research work designed and executed by the authors. There is no conflict of interest associated with this scientific work.

Ethics approval: Not Applicable

Consent for publication: The authors mutually agree to publish this scientific work carried out by them to this journal.

Consent for participate: The authors have consented to participate in the research work carried and publish the technical outcome of the same.

Code availability: Not applicable

Availability of data and material: Specific subset of data to depict the briquetting flow chart and SEM_EDS analysis table provided as the supplementary material

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Authors' contribution

This research work has been completed with the equal contribution from the authors DC, NS, PA and RD along with drafting of this manuscript. The functional property analysis of the fermented oil seed cakes were carried out by the AS. SJ is responsible for designing of the work and evaluation of the results and correcting the manuscript.

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Tables

Table 1 Nutrient characterization of pressed oil seed cakes

Components	Hot pressed	Cold pressed
Carbohydrates (mg/g)	133.56±6.67	122.85±6.14
Proteins (mg/g)	9.48±0.47	13.08±0.65
Reducing sugar (mg/g)	2.11±0.11	2.00±0.12
Free amino acids (mg/g)	1.00±0.05	0.78±0.04

Table 2 Functional properties of cold pressed MOSC after fermentation

Property	Unit	Control	*A. oryzae*	*A. niger*
Bulk Density	g/cm^3	0.58±0.08	0.45±0.02	0.42±0.02
Water Binding Capacity	(g of water retained)/(g of sample)	1.89±0.12	1.52±0.09	1.75±0.10
Oil Binding Capacity	(g of water retained)/(g of sample)	1.00±0.03	1.18±0.07	1.21±0.05
Foaming Activity	%	11.26±1.14	5.00±0.92	6.70±0.43
Emulsifying Capacity	%	42.85±0.62	57.14±0.27	60.45±0.03
Emulsifying Stability	%	35.37±1.22	68.62±1.08	60.42±1.27

Figures

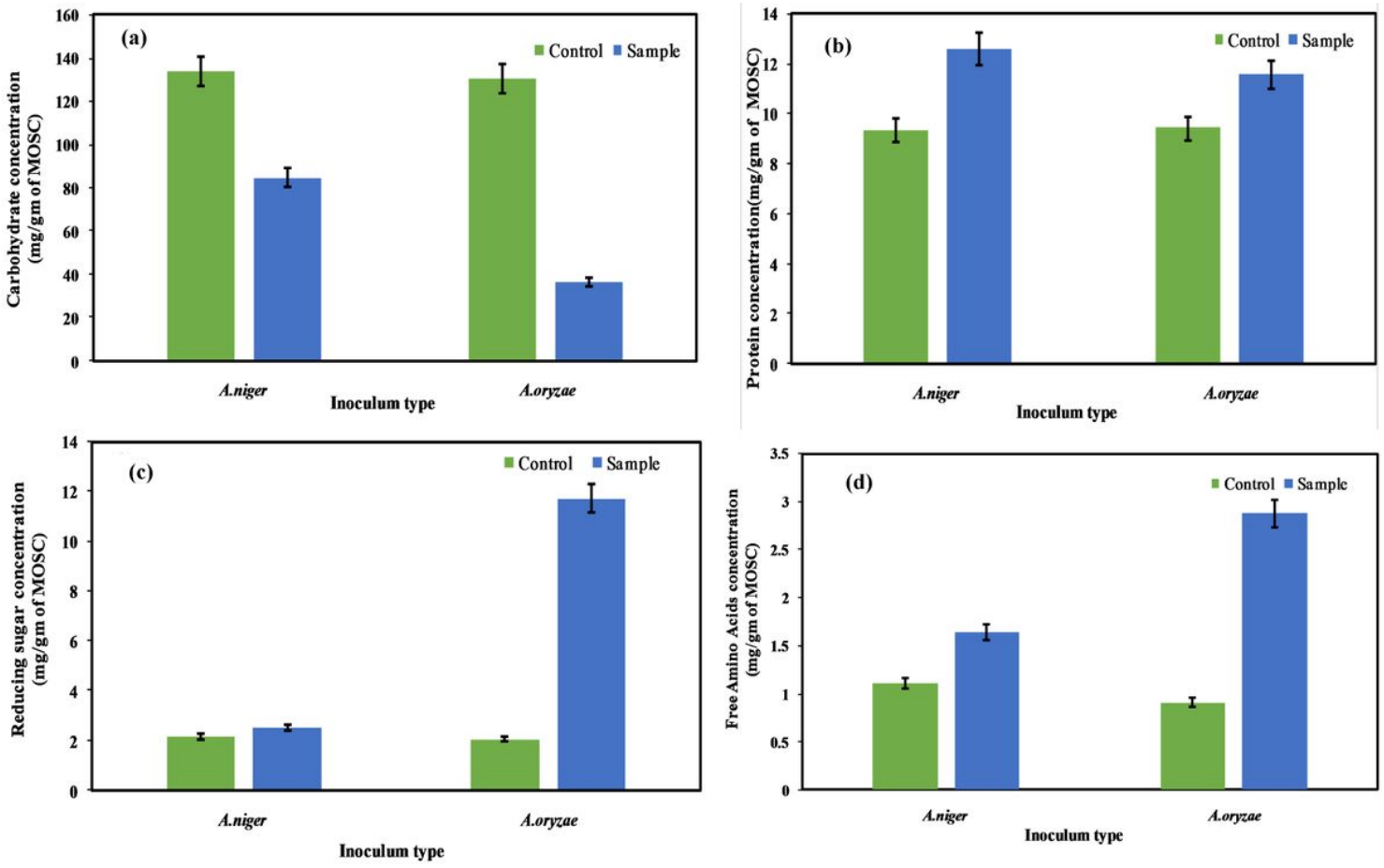

Figure 1

Characteristic changes in the biochemical constituents by A. niger and A. oryzae; (a) and (b) indicate the change in carbohydrate and protein content respectively; (c) and (d) indicate the reducing sugar and free amino acid content respectively

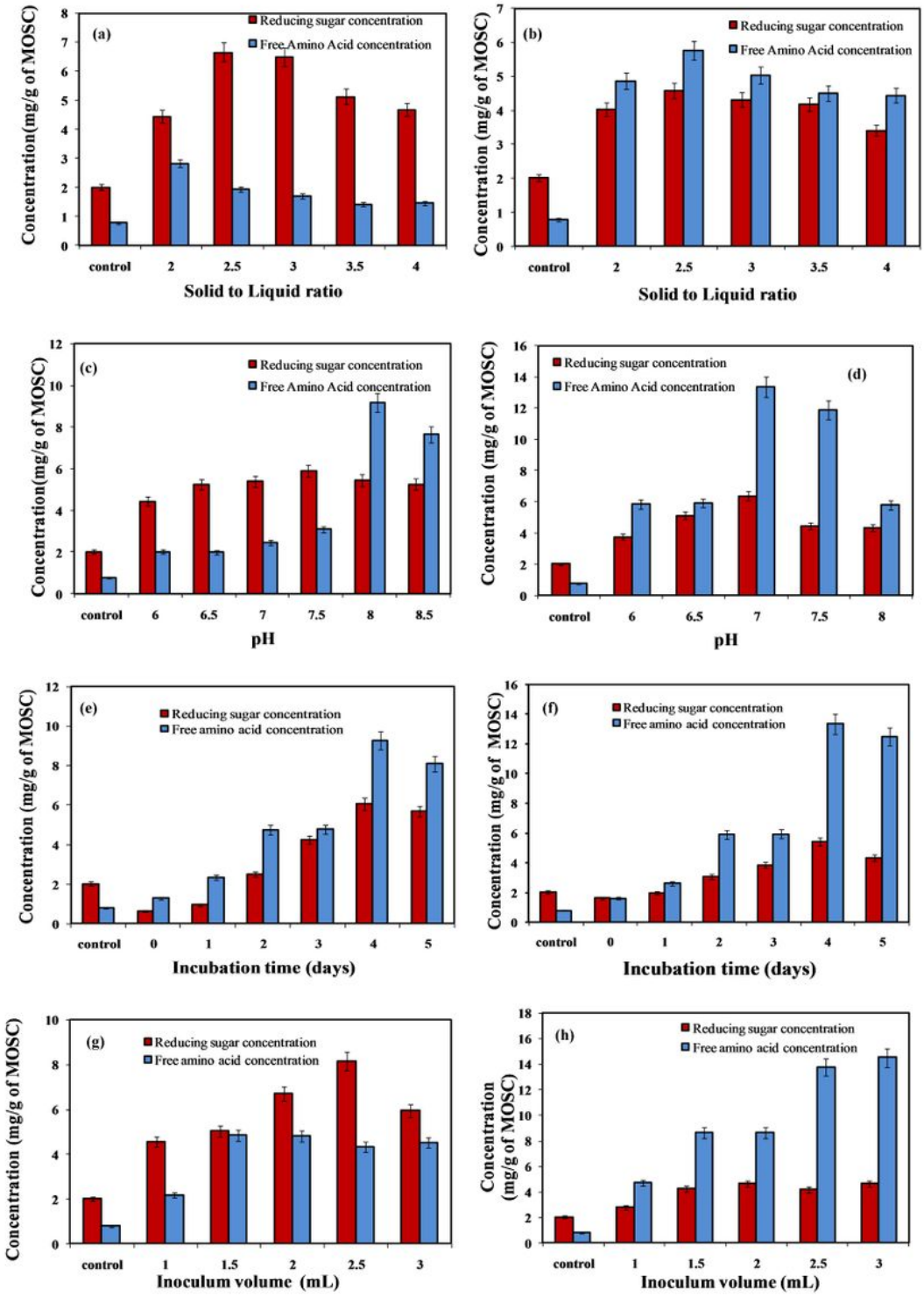

Figure 2

Effect of different process parameters on the fermentative efficacy of MOSC; (a) – Solid:Liquid/A.niger; (b) – Solid:Liquid/A.oryzae; (c) – pH/A.niger; (d) – pH/A.oryzae; (e) – Incubation time/ A.niger; (f) – Incubation time/A.oryzae; (g) – Inoculum volume/A.niger; (h) – Inoculum volume/A.oryzae

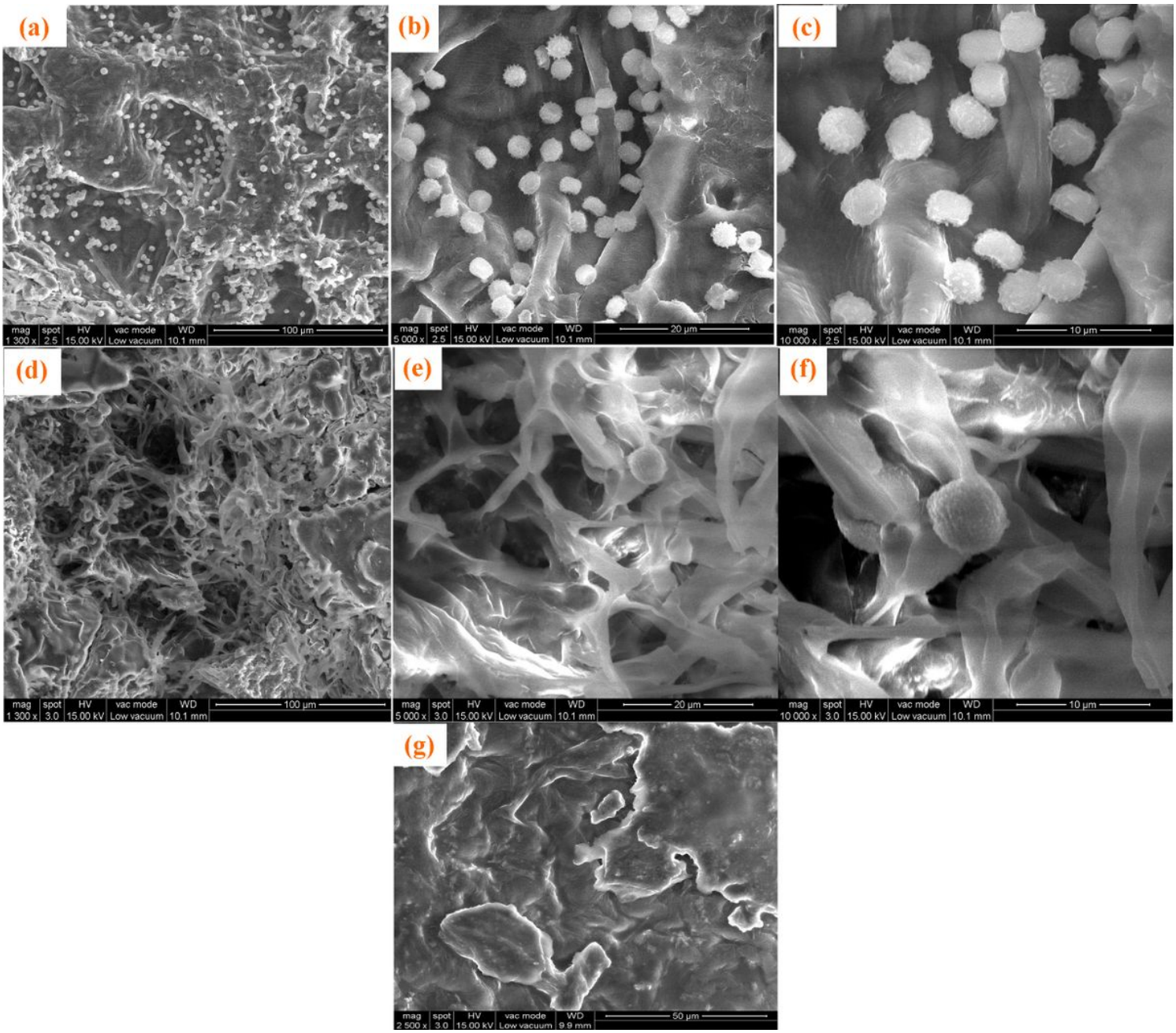

Figure 3

SEM images MOSC briquettes with A. niger (a-1300x; b-5000x; c-10000x) and A. oryzae (d-1300x; e-5000x; f-10000x); g – Control (2500x)

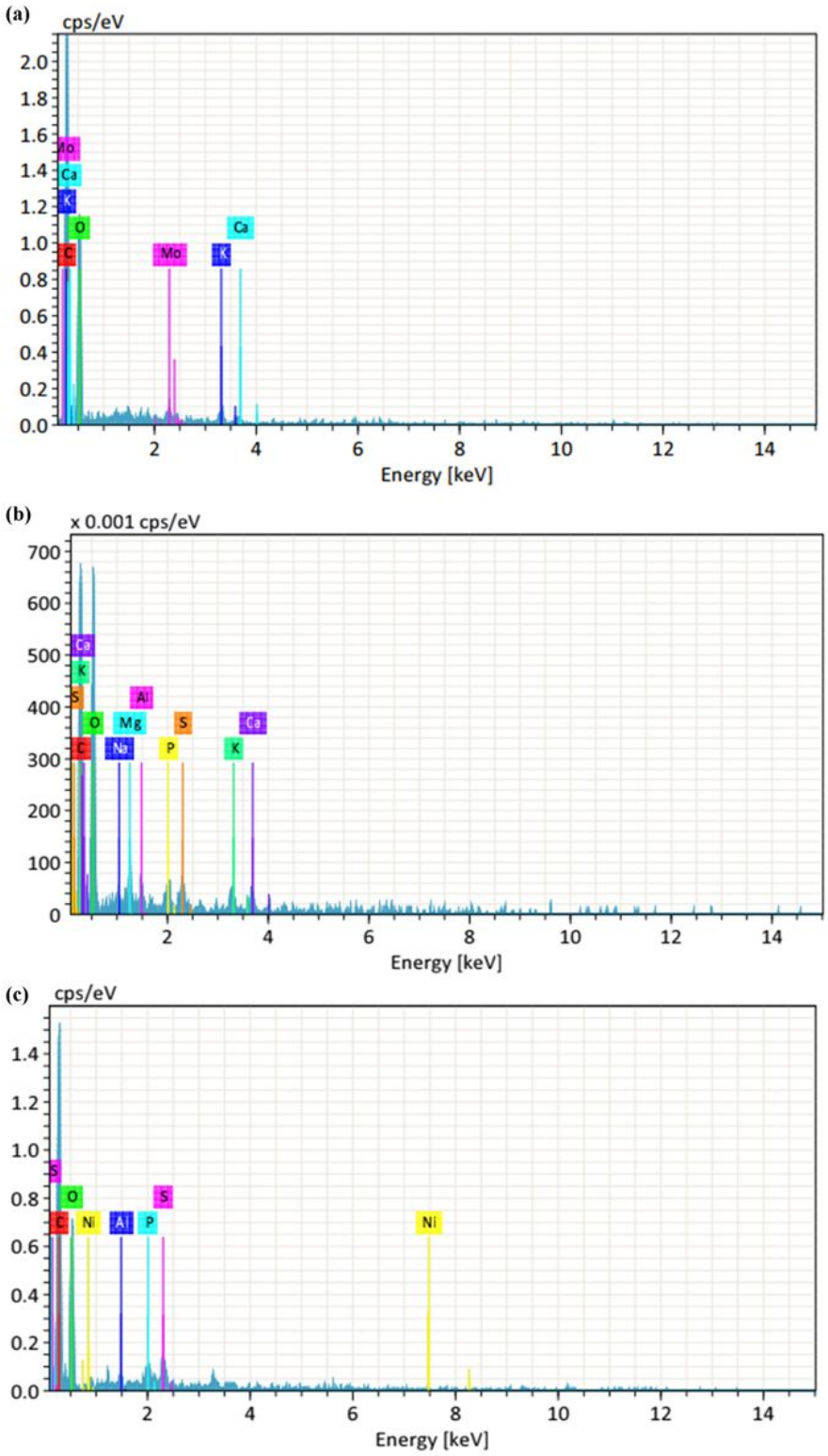

Figure 4

SEM-EDS analysis of (a) unfermented briquettes; (b) A.niger fermented (4 days) briquettes and (c) A.oryzae fermented (4 days) briquettes

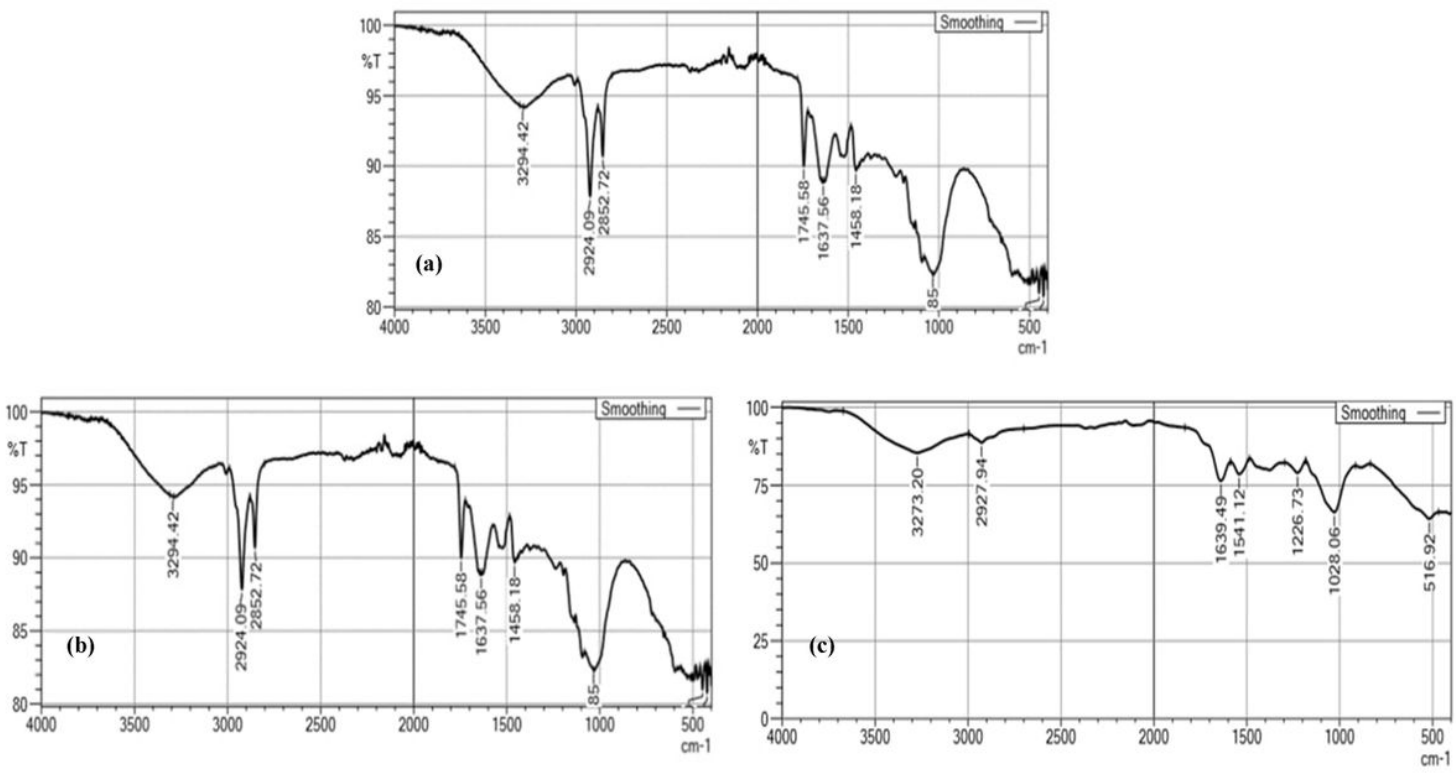

Figure 5

FTIR profile of unfermented and fermented MOSC; (a) – Control; (b) – A.niger fermented and (c) - A.oryzae fermented MOSC

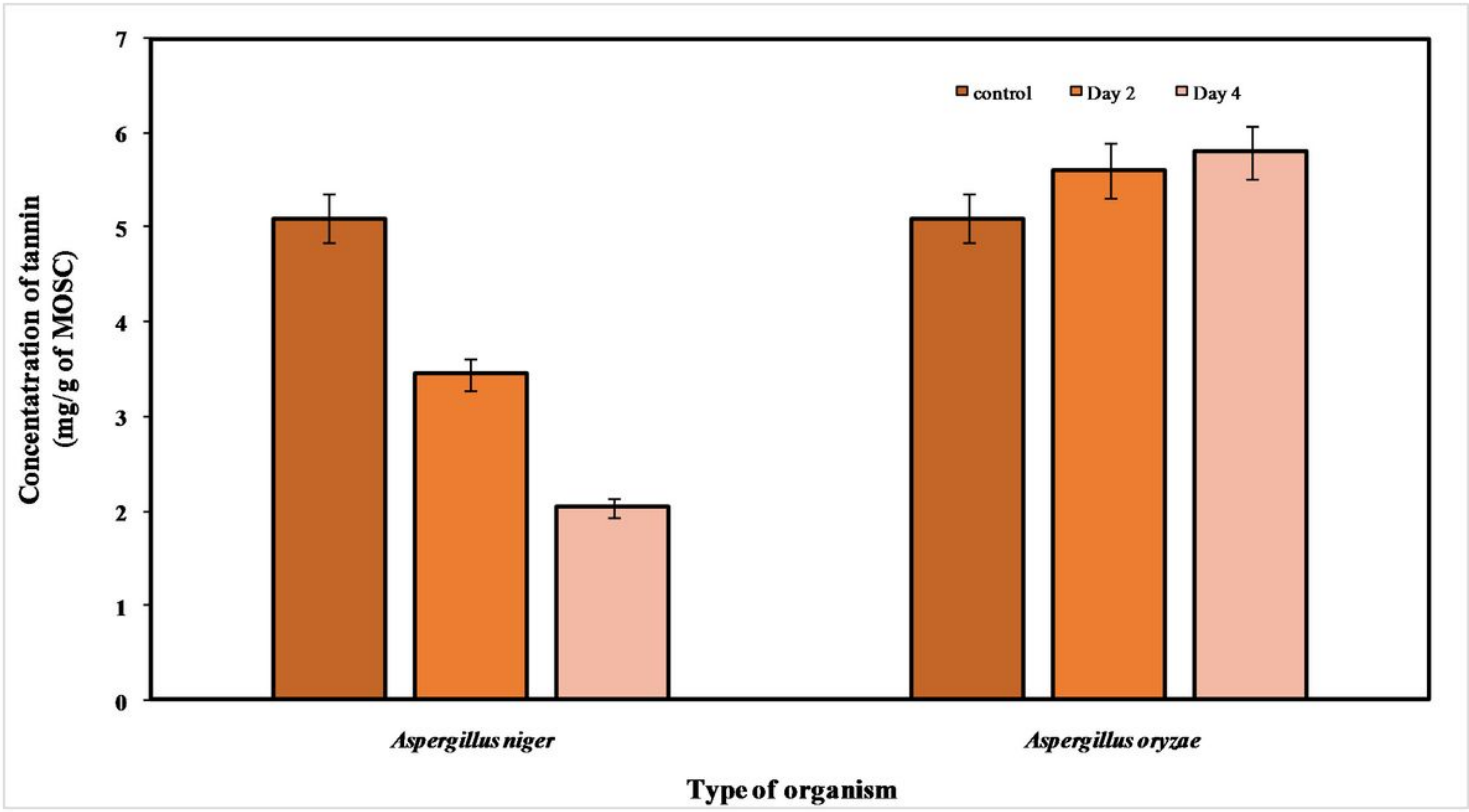

Figure 6

Tannin content in unfermented and fermented (day 2 and day 4) cold pressed MOSC

Supplementary Files

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- Graphicalabstract.tif