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Comparison Of Antioxidant Potential of Some Fermented Oilseed Meals Using *Bacillus subtilis* PTCC 1720

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Abstract

Recently, oilseed meal, a by-product of oil manufacturing, has become the most common animal feed. Regarding the critical role of antioxidants, this study evaluated the effects of fermentation on some press cake antioxidant activities. Sesame, soybean, cotton, corn, and tomato seed meals were used under *Bacillus subtilis* PTCC 1720 submerged fermentation. Antioxidant activity was evaluated using the DPPH inhibition and iron chelation methods. Also, the o-Phthalaldehyde test, thin layer chromatography with two reagents (DPPH and ninhydrin), and SDS-PAGE of proteins were conducted to assess the releasing peptides and anti-oxidant activity relationship. Results showed antioxidant activity in most meals increased significantly after 48 hs of fermentation ($P<0.05$) due to the increase of amino compounds. This activity reached its maximum at 96 hs after fermentation. After 96 h of fermentation, the highest DPPH radical inhibitory activity among the meal samples was observed in black sesame meal (61.2±5.9) and cotton meal (58.5±3.5). Moreover, the soybean sample had a maximum increase in iron ions chelating activity (50.93±4.7) after 96 hs incubation compared to the control. Therefore, the *B.subtilis* submerged fermentation process can raise antioxidant activity in samples, and antioxidant activity can improve meal nutritional value for livestock and poultry feeding.

1. Introduction

Oxidants, like reactive oxygen species (ROS) and reactive nitrogen species (RNS), are generated by lipid oxidation and physiologic reactions that destroy the body and food systems by attacking biomolecules [Aruoma 1994, Kalyanaraman 2013]. A large body of evidence shows that the onset and advance of diseases such as diabetes, metabolic disorders, cancer, arteriosclerosis, and cardiovascular diseases can be caused by oxidative stress [Pizzino et al. 2017]. Antioxidants neutralize free radicals and intensify the immune system's efficiency, thereby protecting the cell from destruction [Nasab et al. 2020]. Antioxidants' vital role in biological systems and the food industry has attracted research interest over the past few years [Aruoma 1994, Kalyanaraman 2013]. Antioxidant activity in plasma can be increased by consuming

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antioxidant-rich foods [Temple 2000]. Furthermore, the shelf life of food products can be extended by 15-200 percent by using antioxidant additives [Tsaliki et al. 1999]. Among antioxidants, natural antioxidants with plant sources are prevalent because they are natural, safe, and cholesterol-free [Zaky et al. 2022].

Bioactive peptides are protein residues with a molecular mass between 0.4-2 KDa and 2-20 amino acid residues. These peptides can be separated through chemical or enzymatic hydrolysis and fermentation. Several studies have shown that these peptides can help human body systems and overall health with their antimicrobial, anti-inflammatory, anti-blood pressure, anti-obesity, and antioxidant properties [Sheih et al. 2009]. Hence, numerous food sources have been studied in this regard, such as algae protein waste, alfalfa leaf protein, whey protein, peanut protein, sunflower protein, rice endosperm protein, etc. [Xie Z et al. 2008, Corrêa et al. 2014, Zhang et al. 2011, Megías et al. 2008, Zhang et al. 2009, Dai et al. 2023].

Oil cakes (press cake), a solid remaining oil extract of any kind of beans (oil beans, soybeans, etc.), are the most common animal food because they contain high amounts of protein and nutritional factors [Tian et al. 2023]. However, their use is sometimes limited due to specific anti-nutrition effects. For example, oligosaccharides were found to enhance digesta viscosity and decrease growth performance in some meals, including soybean meal, by reducing the digestibility of nutrients, especially in monogastric animals and poultry [Song et al. 2008]. However, fermentation can reduce carbohydrates and oligosaccharides, improving animal feeding and growth levels [Chen et al.2013]. Animals are often exposed to oxidative stress conditions at different stages of production, such as heavy metals, environmental stress, disease, high density, as well as transportation, which may suppress the growth performance and animal health [Wang et al. 2022]. It has been found that there is a relationship between the onset of some diseases and the reduction of antioxidant status in livestock. Good animal antioxidant status can positively affect meat quality parameters, improve meat vitamin E content, and reduce meat lipid peroxidation [Corino et al. 2021]. Also, results have shown that oxidative stress in livestock and poultry can affect egg-laying performance, intestinal microbiota, and also reduce ovarian function [Wang et al. 2022].

Fermentation has been found to have potential benefits in expanding the quality of food [Olmos et al. 2014, Tsaliki et al. 1999]. The meal fermentation process not only digests proteins, which improves the absorption of peptides or amino acids for animals, but also increases nutritional value such as phenolic content, vitamins, and pro vitamins while removing some anti-nutritional factors from meals, including oligosaccharides, trypsin inhibitors, phytic acid, and gossypol. Moreover, it can sometimes provide protein hypoallergenic properties [Komatsuzaki et al. 2005]. *Bacillus subtilis* has beneficial properties and is a potential probiotic candidate for producing functional feeds, including growth in the pH range of 5.5-7 (a fast growth rate) and the production of various enzymes and metabolites. Thus, applying this microorganism increases feed degradation, stimulates the immune system, and improves health status [Chantawannakul et al. 2002, Permpoonpattana et al. 2012, Olmos et al. 2014]. Some commercial animal products produced from *Bacillus subtilis* include Vime-Baciflor, BioGrow®, Biozyme®, Proflora, and BioPlus® 2B [Chantawannakul et al. 2002].

Considering the above aspects, this study explores the effect of *Bacillus subtilis* fermentation on the antioxidant capacity of several pressed oil cakes collected from factories and compares the results. The relationship of bioactive peptides with antioxidative activity was also evaluated.

2. Materials and Methods

2.1. Chemical analysis

Oilseed meal (black sesame meal, soybean meal, corn meal, tomato seeds, and cottonseed meal) is

first prepared from oil production units after oil extraction using the cold-press method. Then, the samples' crude nitrogen and protein content were analyzed by AOAC 976.05 International guidelines. A sample of the meals (1.0 g) was simultaneously digested with 10 ml of H_2O_2 (35%), 12 ml of concentrated H_2SO_4 (98%), and 5 g of K_2SO_4 in the presence of selenium dioxide (0.25 g) as a catalyst. The absorbance of the NH3–salicylate complex was read at 660 nm using a spectrophotometer [ISO 1977]. Also, the ash of every meal was determined using ISO-749 methods. Briefly, 2 g of the meals were taken and carbonized by heating on a gas flame. The carbonized material was then ashed in an electric muffle furnace at 550 C until a constant mass was achieved [Mukhtar et al. 2013].

2.2. Bacterial Strain, Culture Conditions, and Preparation of Inoculums

The test organism, *B. subtilis* PTCC 1720, was obtained from the Iranian Research Organization for Science and Technology (IROST). Two colony types from this strain were observed on the agar surface: 1) Circular, entire, low convexity, shiny, and opaque and 2) Irregular, rough, flat, and opaque. After 16S RNA gene sequencing, the two colony types were found to be identical in genus and species (1720 datasheet). The organism on an agar slant was maintained and subcultured monthly. One *B. subtilis* colony was cultivated for 24 h on slant nutrient agar and then transferred to 100 ml nutrient broth pre-culturing medium at 30°C in a rotary shaker. After 18 h, 5ml of medium culture with 1.5×10^8 CFU.ml^{-1} (equivalent to 0.5 McFarland Standard as well as absorbance reading at 620 nm wavelength) was provided as inoculum. Finally, this culture was applied as an inoculum to make various fermented ground foods.

2.3. Fermentation Process

Various amounts of different ground meals comprising about 1 g protein, equivalent to 2.25 g soybean, 3.54 g cotton, 2.65 g tomato seed, 8.11 g corn, and 2.67 g black sesame meal [Church et al. 1983] were added to a 250 ml flask culture contains 100 ml culture medium of 0.1% K_2HPO_4 and 0.05% $MgSO_4.7H_2O$. They were then autoclaved for 20 min at 121°C and 15 psi. Finally, 2% filtered glucose and 1% inoculum were added, and the sample was incubated for 96 hs at 30°C. Next, cultivation sampling was conducted at various times (0, 6, 12, 24, 48, 72, and 96 h). Afterward, to inactivate the protease, the supernatant of the samples was heated for 10 min at 100°C and then centrifuged at 8854×g for 20 min at 4°C.

2.4. Determination-free amino groups

Peptides or amino acids were analyzed by measuring free amino groups. The reaction between free amino groups and o-Phthalaldehyde (OPA) in the presence of β-mercaptoethanol leads to the formation of a compound that can be detected at a wavelength of 340 nm in a spectrophotometer. Briefly, 10 µl of the supernatant of the fermented samples was first thoroughly mixed with 1 ml of OPA reagent and incubated for 4 minutes at room temperature. Then, after measuring the absorbance of the samples at 340 nm wavelength, the amount of free amino groups was determined using the standard curve of serine amino acids with specific concentrations [Nielsen et al. 2001].

2.5. Antioxidant Properties

2.5.1. 1,1-Diphenyl-2-Picrylhydrazyl (DPPH)

The radical scavenging potential was obtained using the modified technique from [Shariati et al. 2019]. First, 0.05 ml of supernatant was added to 1 ml DPPH methanolic solution (0.5 M). Then, the mixtures were incubated at room temperature in the dark for 60 minutes, and their absorbance was read at 517 nm using a Shimadzu UV-VIS spectrophotometer. The results were expressed as a percentage of free radical scavenging capacity.

$$\%\ \text{DPPH} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

$A_{control}$ is the absorbance of the control (a mixture of DPPH reagent with water instead of the

sample), and A_{sample} is the absorbance of the test sample.

2.5.2. Iron chelating potential assay

The iron-chelating potential of samples was conducted using an adjusted method from [Zhu K et al. 2006]. The supernatant of fermented meal samples (0.05 ml) was blended with distilled water (1 ml) and FeCl2 (32 µl, 1 mM), and after 30 seconds, ferrozine (0.67 µl, 5 mM) was added and blended. The mixture was allowed to stand at room temperature for 20 min, and then the absorbance of samples in 562 nm was measured. Synthetic metal chelator EDTA was applied as the positive control.

2.6. Thin layer chromatography (TLC)

After 24, 48, 72, and 96 h of fermentation, the samples were used twice for thin layer chromatography (TLC silica gel 60 F254 - 1.05554.0001). The mobile phase employed for this analysis consisted of a blend of n-butanol, water, and acetic acid glacial in a ratio of 1.5:1:1, as described in [Mohammad et al. 2012]. One of the TLCs was sprayed with the ninhydrin reagent (ninhydrin 0/25% in acetone), and the other was sprayed with the DPPH reagent (1M DPPH in methanol) and left at 80 – 100 °C for 5 min, allowing the spots to appear. The reagent reaction was visualized in the presence of two specific reagents. Serine amino acid 1% was used as a control in each TLC.

2.7. Protein Profiling by SDS-PAGE

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) 15% [Sambrook et al. 2006] was used to analyze the protein degradation in the meal samples during fermentation. The samples were mixed with the 5X sample treatment buffer (1% b-mercaptoethanol, 250 mM Tris-HCL buffer pH 6.8, 0.1% bromophenol blue, and 5% SDS) and boiled for 8 min. After electrophoresis, the gel was painted with Coomassie blue, similar to the technique used in [Gregan et al. 2007].

2.8. Statistical analysis

All the testing performed in this research was repeated three times, and the mean and SD indicators were used to show the distribution and summation of the data. The results were investigated using one-way ANOVA statistical tests using GraphPad Prism version 8 software, and the significance level was assessed at a P-value < 0.05.

3. Result and discussion

3.1. Proximate composition of Oilseed meals

Results regarding the protein, nitrogen, and ash content of oilseed meals have been listed in (Table 1). The results show that the highest protein percent among meals was presented by soybean meal at 44.33 ± 0.75%, and the lowest was observed in corn meal at 12.33 ± 0.50%. In contrast, a previous study found 37.69% soybean protein and 4.29% ash [Ruth et al. 2018]. This difference could be due to the different sources of soybeans and also the measurement method. However, another study that examined six samples of cotton seeds from various companies in Tanzania showed that the protein content of these samples was between 24 and 48% [Jagadi et al. 1987], consistent with the results of our research.

3.2. Determination of free amino acids in meals using the OPA method

(Fig 1) shows free amino acids in oilseed meals at different fermentation times. The OPA test of the samples showed that the amount of free amino groups slightly decreased during the initial 12 hs, and this amount increased in most of the meal samples as the fermentation time increased up to 96 h. However, in some meal samples, such as tomato seed and soybean samples, the free amino groups remain constant after 72 hs. In general,after 96 hs fermentation, the largest increase in the free amino group was shown in the black sesame seed sample (1.54±0.01), followed by cotton, soybean, and tomato seed. Corn meal had the smallest increase in the free amino group at the end of incubation. The degree of hydrolysis (DH) indicates the extent of peptide bond cleavage in the

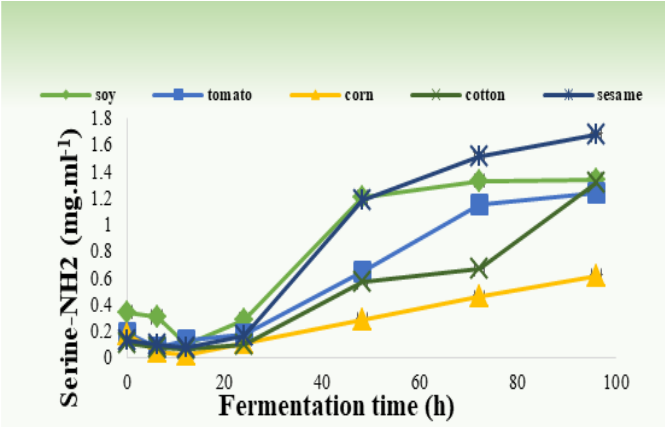
Table 1: Proximate composition of Oilseed meals

Meal kind	Nitrogen (%)	Protein (%)	Ash (%)	Ash of dry weight (%)
Soybean meal	7.15±1.02^{a}	44.33±0.75^{a}	5.82±0.31^{ab}	6.47±0.43^{ab}
Cotton meal	4.63±0.87^{ab}	28.19±0.57^{ab}	3.48±0.38^{ab}	3.72±0.16^{ab}
Tomato seed meal	5.66±0.45^{ab}	37.63±0.93^{ab}	5.42±0.28^{ab}	5.73±0.21^{ab}
Corn meal	2.11±0.20^{b}	12.33±0.50^{b}	1.87±0.10^{a}	2.03±0.06^{a}
Black sesame seed	5.64±0.60^{ab}	37.32±0.47^{ab}	11.91±0.29^{b}	12.54±0.44^{b}

Various letters in each column display significant differences between the data.

protein substrate by a proteolytic agent: a higher value corresponds to a greater release of amino groups. Proteolytic enzymes break down large intact proteins into smaller polypeptides and intermediate peptides [Gänzle et al. 2008]. As most small peptides and free amino acids can be directly absorbed by the digestive system, hydrolysis plays a significant role in influencing the nutritional value of plant-based food products. It is important to note that the activity of proteinases and peptidases depends on time, so prolonged fermentation contributes to increasing DH [Feng et al. 2024].

Figure 1: Effect of fermentation time on free amino groups of meals.



3.3. Antioxidant Capacity of Oilseed Meals

Compounds with antioxidant properties can exert their effects through various mechanisms. The antioxidant potential of oilseed meals was measured using DPPH radical scavenging activity and iron ion chelation activity (Fig 2 and 3). The results showed that after 48 h of incubation, the DPPH radical scavenging activity in the samples increased. The sesame meal sample (61.2 ± 5.9) and the cotton meal sample (58.5 ± 3.5) exhibited the highest increase in DPPH radical scavenging activity among the different meal samples. Additionally, among all the meals, the soybean sample showed the greatest increase in iron ion chelation activity (50.93 ± 4.7) after 96 h of incubation compared to the control group.

Figure 2: DPPH radical scavenging activity of black sesame meal, soybean meal, corn meal, tomato seeds, and cottonseed meal during 96 h fermentation.

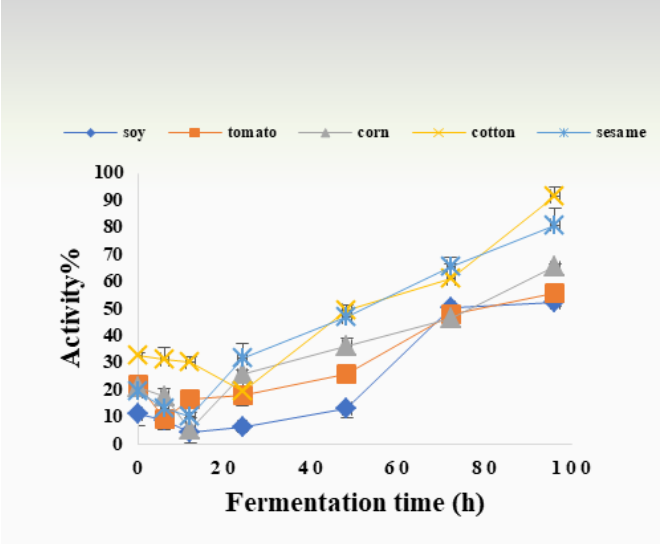
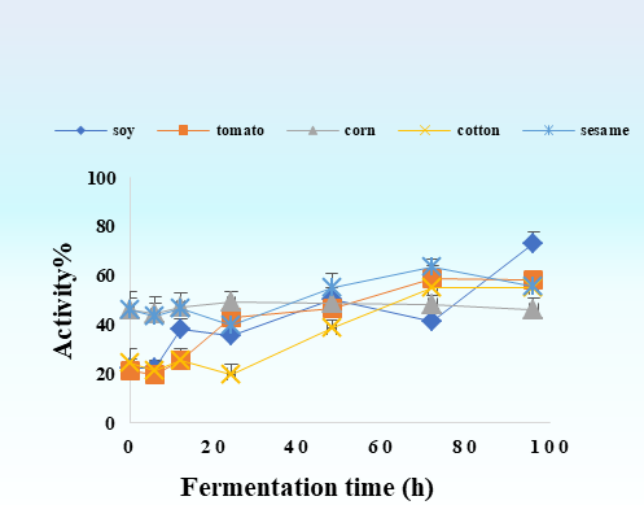


Figure 3: Iron chelating activity of black sesame meal, soybean meal, corn meal, tomato seeds, and cottonseed meal during 96 h fermentation.



According to Figures 2 and 3, it can be concluded that during the fermentation of *B.subtilis*, the antioxidant potential of oilseed meals increased. A study comparing the antioxidant capacity, total phenols, and flavonoids of black and yellow soybeans fermented with *B. subtilis* with unfermented soybeans concluded that fermentation increased the total phenolic and flavonoid contents as the potential of the anti-DPPH radical antioxidant and iron-reducing antioxidant power (FRAP) of soybean extract increases with fermentation [Dajanta et al. 2013]. Also, a study on the effect of fermentation with *Bacillus subtilis* on black soybean extract under different solvents and the trace of fermentation on changes in total phenol content, flavonoid, and antioxidant activities concluded that fermentation increases the total phenolic, flavonoid content, and black soybean extract's antioxidant potential [Juan et al. 2010]. The results of the present research are consistent with the above studies. According to Figure 1, the highest increase of free amine groups is observed in the case of black sesame seeds (1.54 ± 0.01), followed by cotton, soybean, tomato seeds, and corn, and the antioxidant activity in the DPPH test (fig 2) has the highest increase in the black sesame and cotton meal samples, followed by corn meal, soybean meal, and tomato seed. In the case of most of the samples, the increase of amine groups was proportional to the antioxidant activity. In general, iron chelating power at different times did not follow a specific pattern, unlike DPPH radical scavenging activity, which had an upward trend, possibly due to the creation of peptides with different molecular sizes during fermentation [Je et al. 2009].

The increase of antioxidant activity in fermented products may also depend on other factors. One of these factors is the glycosylation of phenolic glycosides during the fermentation process, which causes the release of more phenolic aglycans and, as a result, enhances antioxidant activity. Also, the release of polyphenols from protein complexes can help increase the antioxidant capacity of fermented products [Chen et al. 2017]. Some amino acids, such as tyrosine, cysteine, and methionine, are known as effective factors in antioxidant activity. For example, the increase in the antioxidant capacity of fermented rapeseed meal is related to the increase in these amino acids [Wang et al. 2022]. Anti-nutritional factors refer to chemical entities in plant products that negatively affect the digestibility of proteins or other macronutrients such as starch and, hence, their bioavailability. The reduction of anti-nutritional factors during fermentation can indirectly increase the accessibility of enzymes to proteins, which in turn increases protein digestibility. Previous studies have shown that post-fermented soybean meal can strengthen intestinal barrier function, weaken undesirable intestinal mucosal immune response, and reduce the incidence of diarrhea in animals [Feng et al. 2024].

3.4. Thin layer chromatography (TLC)

TLC was conducted to verify the free amino groups' correlation with DPPH scavenging activity. Results showed that spots created on the same dotted TLC were approximately similar (Fig 4). This data suggested that free amino groups in reaction with Ninhydrin were related to the DPPH scavenging activity. However, some spots in the DPPH reaction TLC did not exist in the ninhydrin reaction TLC. Moreover, before fermentation and 24 h after fermentation, the upper spots at 72 and 96 hs were not totally clear, and there were some spots on the DPPH reaction TLC.

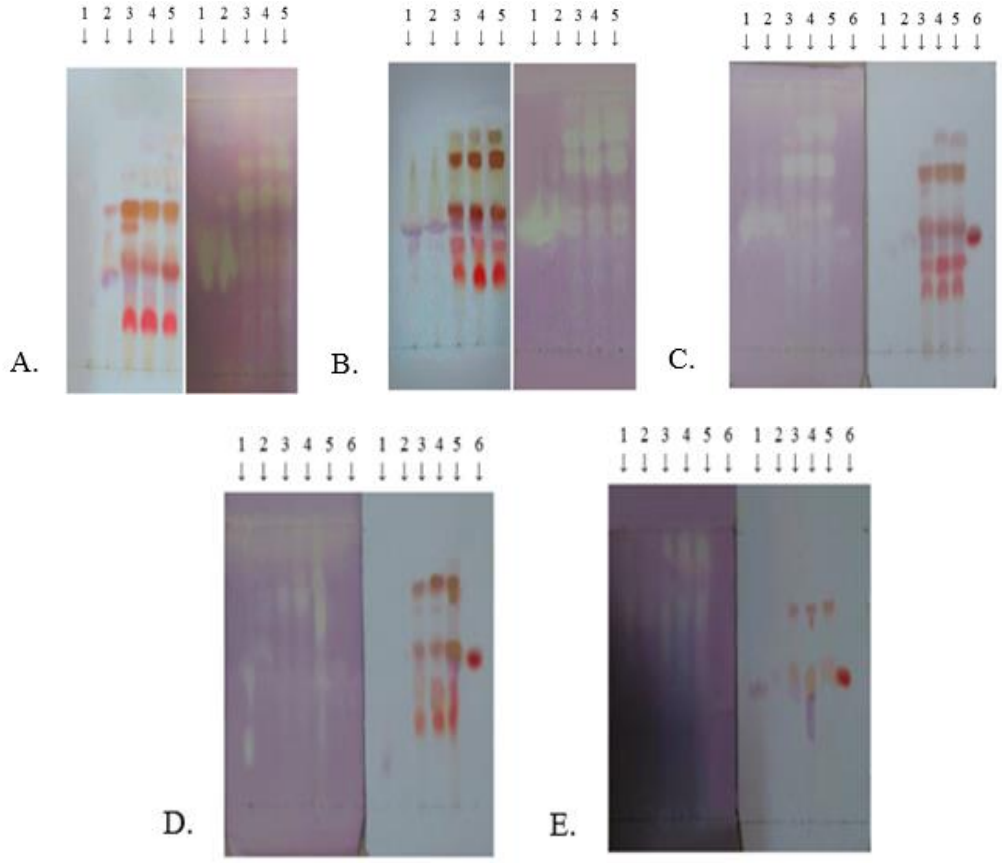


Figure 4: TLC of meals, A: TLC of soybean meal, B: tomato seed meal, C: black sesame meal, D: cottonseed meal, and E: corn meal. Lane 1: before fermentation, 2: 24, 3: 48, 4: 72, 5: 96 hs after fermentation, and 6: Serine amino acid. The figure with a purple background is the result of spraying the Ninhydrin reagent, and the figure with a white background is the result of spraying the DPPH reagent.

It is worth mentioning that fermentation leads to the release of various compounds with antioxidant activity. This collection includes some amino acids, small peptides, and phenolic or colored compounds. However, considering the highest antioxidant activity in the present study was observed when there were the most peptide compounds and free amine groups in the environment, and also considering the bands formed in TLC, the antioxidant compounds in the DPPH test on TLC paper (Fig 4) can be attributed to peptide compounds and protein and peptide derivatives. Many studies have reported that phenolic compounds increase during fermentation. Since phenolic compounds are usually attached to carbohydrates, fatty acids, and proteins [Komatsuzaki et al. 2005], fermentation can break down these compounds and increase phenolic compounds, thus increasing antioxidant activity. This becomes an oxidant of plant seeds, an advantage of fermentation compared to the use of pure enzymes.

The relationship of the antioxidant activity increasing with the free amino groups was confirmed qualitatively by comparing two identical dotting thin layer chromatography in the presence of two specific reagents, and the DPPH radical scavenging quantitative test revealed a correlation between free amino groups and DPPH radical scavenging. Two samples of black sesame meal and cotton meal showed the highest increase in DPPH free radical inhibition activity due to the highest increase in the free amine group after 96 hs of fermentation. A graph of the two black sesame and cotton meals is shown for better comparison (Fig 5 and 6). The relationship

between antioxidant activity and free amine groups is also depicted in Figures 5 and 6. A study showed that increasing peptides increases antioxidant activity [Zhang et al. 2011], similar to the results of the present study.

3.5. Profiles of cell growth and pH

In this study, oilseed meals were used as the sole nitrogen source, so pH measurement and colony counting were performed to evaluate the meal nitrogen and bacterial growth. Results showed that the *B. subtilis* in soybean meal and sesame seed grew better than in the other meal mediums.

After 48 h fermentation, the exponential growth of bacteria decreased or stopped in almost all mediums (Figure 8). However, after 48 hs of fermentation, the exponential growth of bacteria decreased, and free amino groups increased in almost all mediums (Figures 5 and 6).

Figure 5: The relationship between antioxidant activity and free amino groups in cotton meal during fermentation.

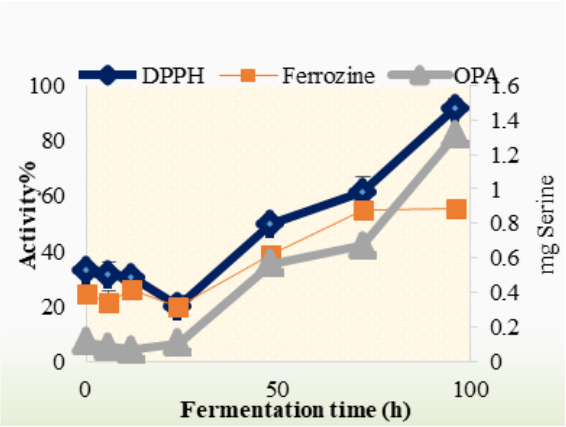


Figure 6. The relationship between antioxidant activity and free amino groups in Black sesame meal during fermentation

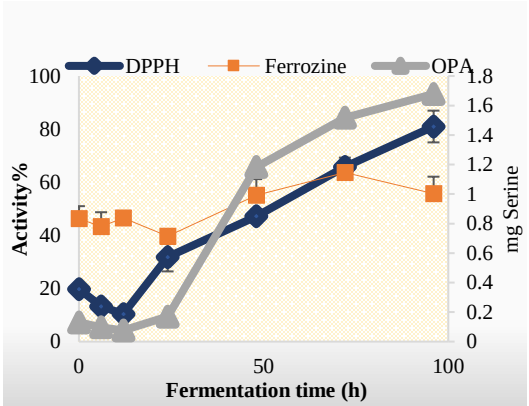


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Figure 7: Changes in pH in the black sesame meal, soybean meal, corn meal, tomato seeds, and cottonseed meal during 96 h fermentation.

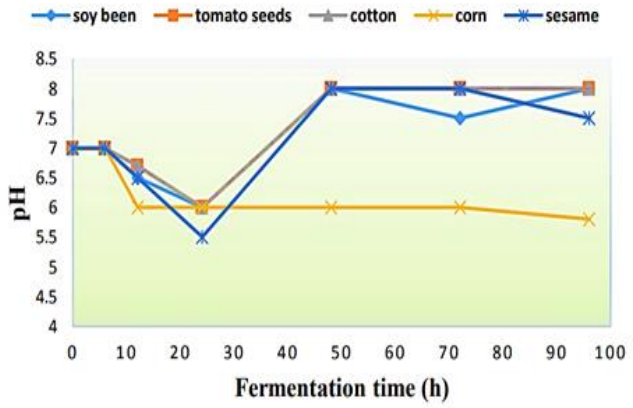
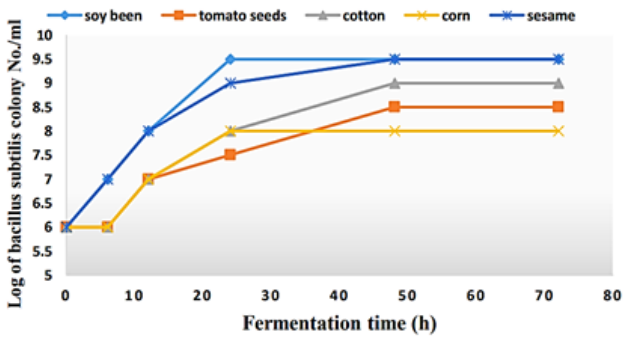


Figure 8: Changes of colony forming unit of black sesame meal, soybean meal, corn meal, tomato seeds, and cottonseed meal during 96 h fermentation.



The results shown in (Fig 7 and 8) indicate an increase in the population of *B. subtilis* in this culture. Since the only source of nitrogen in this culture is the oilseed meal, it can be concluded that bacteria can grow, use, and break down the proteins of the oilseed meal. The increase in pH is the result of bacterial activity, the breakdown of proteins, and the release of ammonia observed over time [Terlabie et al. 2006]. According to (Fig 8), *B. subtilis* enters the stationary phase at 24 hours. Several studies have shown that *Bacillus* species, such as *B. subtilis*, produce extracellular proteinases in the late exponential and stationary stages of growth. It has also been shown that when complex organic compounds are used as carbon or nitrogen sources, cells in the stationary and early phases can maintain a certain level of metabolic activity and RNA content, leading to a continuous accumulation of proteases [Sun et al. 2023].

3.6. Protein degradation

Figures 9 and 10 present the SDS-PAGE analysis of oilseed meals. After 48 h fermentation, peptides and protein components with a molecular weight lower than 17 kDa increased, and after 96 h fermentation, large proteins were almost decomposed with no significant band seen. This is observed due to the production of protease in the stationary phase and the entry of bacteria into the stationary phase 24 hs after fermentation. Large molecular weight protein bands disappeared during fermentation, and lower molecular weight protein bands increased. The proteins in the oil meal were broken into smaller molecules due to strong proteolysis during fermentation with *B.subtilis*, which reduced the larger size of proteins. Figures 9 and 10 show that fermentation caused the breaking and hydrolysis of large proteins. Moo Chang-kook et al. used *B.subtilis* as the starting organism for solid-state fermentation to increase the nutritional quality of soybean meal as animal feed; they observed that fermentation decreased the molecular mass of soybean meal proteins [Kook et al. 2014]. The above results are similar to the results of the present research.

Figure 9: SDS PAGE of A: black sesame meal and B: cottonseed meal (lane 1: standard molecular weight, lane 2-8, before fermentation, 6 h,12 h, 24 h, 48 h, 72 hs, and 96 hs after fermentation respectively).

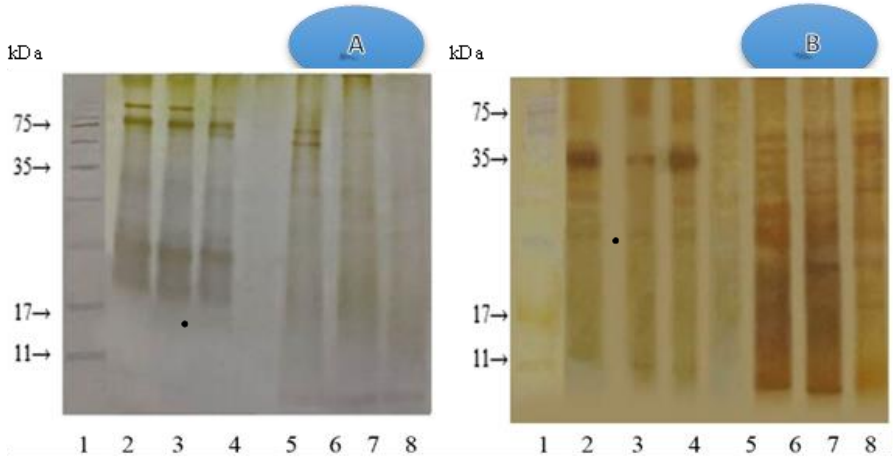
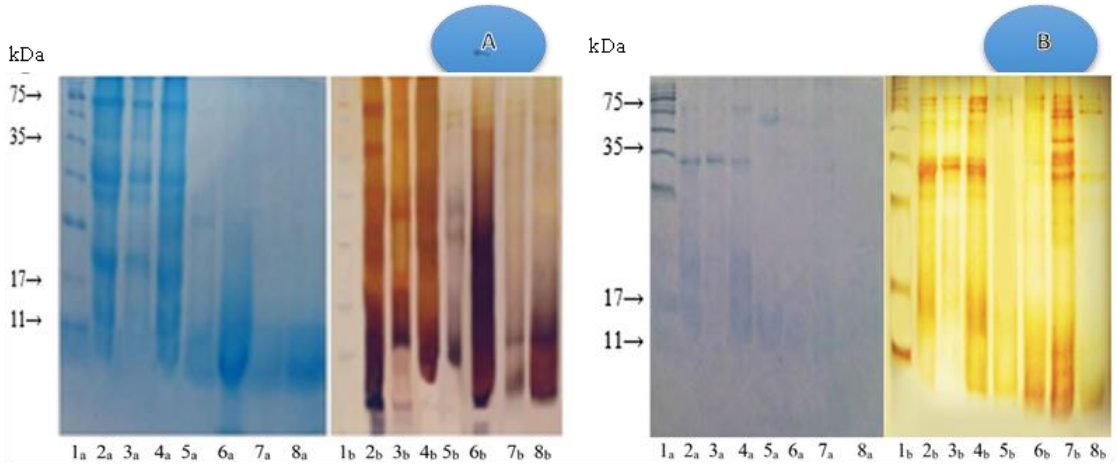


Figure 10: SDS PAGE of A: soybean meal and B: tomato seed meal. (lane 1a: standard molecular weight, lane 2a-8a, before fermentation, 6,12, 24, 48, 72, and 96 hs after fermentation, respectively, by Coomassie brilliant blue G-250 staining). (lane 1b: standard molecular weight, lane 2b-8b, before fermentation, 6,12, 24, 48, 72, and 96 hs after fermentation, respectively, by silver staining)



Chen et al. (2020) investigated the effect of soybean fermentation using the *Eurotium cristatum* fungal strain to determine the trace of fermentation on the amount of total phenolics, isoflavone compounds, and antioxidant activity of soybean during different periods of fermentation. They concluded that total phenol, isoflavone, and antioxidant activity increased during fermentation. Other studies have investigated the effect of *B. subtilis* fermentation (due to its potency to inhibit the growth of harmful microorganisms and its great protease activity on plant seeds such as rapeseed meal and wheat gluten), and the results showed that the concentration of soluble peptides and acids free amino after fermentation and the concentration of small molecular peptides as well as the antioxidant activity of these seeds increased after fermentation [Wang et al. 2022, Zhao et al. 2023]. Inoverall, based on the high value of biofunctional properties in samples, it could be advised to convert rapeseed oil press residues into value-added foodstuffs to achieve a health-promoting food source.

4. Conclusion

According to the obtained results, the microorganism *B. subtilis* can grow and hydrolyze the proteins of plant seed meal as the only source of nitrogen, in most culture media containing plant seed meal;. This study observed the relationship between the increase of free amino groups and the antioxidant activity of meals. After 96 h of fermentation, sesame and cotton meals showed the highest increase in antioxidant activity compared to other samples, and the lowest increase in antioxidant activity was seen in corn meal. However, *B. subtilis* fermentation was not found to be a good choice for hydrolyzing corn meal, but other enzymatic treatments can be employed instead. As a source rich in protein, plant seed meal is a cheap and available source to obtain bioactive peptides, which have been shown to promote health, prevent, and even treat some diseases.

Conflict of interest

The authors declare no conflicts of interest.

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Ethical approval

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