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Solid-State Fermentation of Corn and Soybean by *Bacillus subtilis* Egyptian Isolate

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ABSTRACT

The major ingredients of poultry and animal diets are corn and soybean. In the current study, *Bacillus subtilis* isolate was used to ferment soybean and corn. Total phenolic content, antioxidant activity, antimicrobial activity and some nutrients of fermented soybean and corn have been investigated. Antioxidant activity and phenolic content of fermented corn and soybean significantly increased after fermentation of soybean while decreased in fermented corn. Fermented soybean and corn exhibited varying degrees of inhibitory activity against eight pathogenic microorganisms. The amount of crude protein and ash percentage of both fermented corn and soybean increased while carbohydrate and oil content decreased during fermentation of both substrates. As a consequence, fermented soybeans should be added to animal feed.

INTRODUCTION

Solid-state fermentation is defined as process used to produce some metabolites generated by microorganisms grown on a solid such as soybean or wheat bran for industrial applications, that process consists of depositing a solid culture substrate such as soybean or wheat bran in a temperature-controlled room with low water level (Raimbault, 1980; Pandey, 2003). *Bacillus subtilis* is a Gram-positive bacterium, facultative anaerobe, endospore-forming rod and catalase positive. (Nakano and Zuber, 1998).This species is commonly found in the upper layers of the soil, in human gut and in higher elevation (Hong *et al.*, 2009, Sudhagar and Reddy, 2017). *B. subtilis* ferments traditional foods and changes nutritional properties of these foods (Ashok, *et al.*, 2010). Corn (*Zea mays*) and soybean (*Glycine max*) are the most commonly used in animal feed. Soybean is source of protein and energy of animal diet (Li *et al.*, 1990).

Previous studies have shown that the positive role of fermentation on the nutritional value of animal feed by increasing the bioavailability of nutrients. In addition, the growth performance of animals and the gastrointestinal health of animals (Song *et al.,* 2010). Plant wastes have a potential source of bioactive compounds and antioxidants, which could be used as functional ingredients in animal feeds (Fontana *et al.,* 2013). Antioxidants donate one of their electrons or hydrogen to free radicals, stopping their chain reaction (Kaur and Kapoor, 2001). Supplementation of animal feeds with phenolic compounds may have positive effects on animal gut health by improving antimicrobial activity (Ignat *et al.,* 2011). The aim of this study is concerning with the impact of solid-state bacterial fermentation on components and bioactivities of corn and soybean.

MATERIALS AND METHODS

1. Microorganism and Basal Substrate:

Bacillus subtilis isolate was obtained from Botany and Microbiology department, faculty of Science, AL-Azhar University, Assiut branch was stored at 4°C and sub-cultured at 30°C for 24 h periodically. Table .1 shows the confirmation of identified *B. subtilis* by morphological and physiological means according to Bergey *et al.,* (1984)

Table 1. Characteristics of *B. subtilis* isolate

Test	Result	Test	Result
Gram stain	+	Hydrogen sulfide production	_
Shape	Rod	Indole production	_
Endospore	+	Acid from mannitol	+
Motility	+	Nitrate reduction	+
Catalase	+	Oxidase	+
Citrate (simmons)	+	Phenylalanine deaminase	_
Acid from sucrose	+	Acid from lactose	_
Gas from glucose	+	Urea hydrolysis	_
Gelatin hydrolysis	+	Voges-proskaeur	+
Acid from glucose	+		

+: positive -: negative

2. Inoculum Preparation:

Bacterial suspension (1ml) was prepared by inoculated 100 ml suspension of nutrient broth (5g/100ml) after sterilization at 121°C for 15 min. The cultures were shaken overnight at room temperature. From this 5 ml were used as starter in the following experimental work.

3. Preparation of Solid-State Fermentation:

Soybean and corn obtained from a local market were ground to pass a 3 mm sieve and addition of water before autoclaving 2% (v/w), *Bacillus subtilis* activated on nutrient broth for 16 h for solid-state fermentation (SSF) as described Limón *et al.,* (2015). The slandered inoculum of *Bacillus subtilis* added to ground soybean and corn mixed carefully under sterile conditions and incubated at 37° C for 24 hrs. All experiments were performed in triplicate.

4. Determination of total Phenolic Content:

Samples were prepared and extracted by adding 25 ml of 50 % ethyl alcohol on 0.5gm of each sample (fermented and non-fermented), then agitation for two hours, followed by centrifugation at 3000 rpm for 20 min. Total phenolic compounds (TPC) were examined using modified Folin– Ciocalteu method (Jaramillo-flores *et al.,*2003). The modification was achieved by mixing 900 µL of 10 fold

Folin–Ciocalteu phenol reagent (diluted 1:10 with distilled water) into 100 µL aliquot of ethanolic extract and allowed to stand for 5 min at room temperature; then 0.75 µL of 7% sodium bicarbonate solution was added to the mixture, vortexes for 30 s, and allowed to stand for 90 min at room temperature. The absorbance of the prepared samples was checked at 725 nm using a spectrophotometer (6505 UV/Vis, Jenway LTD., Felsted, Dunmow, UK). A calibration curve of Gallic acid (ranging from 0 to 1.00 mg/mL) was prepared and tested. All values were expressed as mean (mg Gallic acid equivalents/g of Dray weight for 3 replications.

5. Determination of Antioxidant Activity:

Samples were extracted using methods described by (Zielijski *et al.*, 2008). The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay was carried out according to the method described by Lee *et al.*, (2003), with some modifications. The stock reagent solution (10-3Mol) was prepared by dissolving 22 mg of (DPPH) in 50 ml of methanol and stored at 20°C until use. The working solution (6 x 10^{-5}Mol) was prepared by mixing 6 mL of stock solution with 100 mL of methanol to obtain an absorbance value of 0.8±0.02 at 515 nm, as measured using spectrophotometer. A 0.1 ml of extracted samples mixed with 3.9 ml of DPPH solution. Vortex for 30 s and left to react for 30 min, the absorbance was measured at 515 nm. The control without addition of extract was also analyzed. Scavenging activity was calculated as follows: DPPH radical scavenging activity (%) = [(Ab control - Ab sample) / Ab control] X 100 Where Ab is the absorbance at 515 nm.

6. Determination of Antimicrobial Activity:

All samples were minced and extracted with 10 times methanol (1:10) (w/v) at room temperature for 5 h (repeated three times) and then filtered through Whatman No. 4 filter paper. The methanol extracts of each sample were concentrated at 40 °C under vacuum and freeze-dried. Antimicrobial activity was evaluated against eight microorganisms (source: Department of Microbiology and Immunology, Faculty of Veterinary, Sohag University, Egypt) using agar well diffusion assay (Schillinger, 1989).

7.Determination of Chemical Composition of Fermented and Non-Fermented Samples:

Moisture, protein, fat and ash contents were determined according to official methods as described in A.O.A.C. (2000). Carbohydrate content estimation by Phenol sulphuric acid method (Masuko *et.al*, 2005).

8. Statistical Analysis:

The studied data analyzed by using SAS program (SAS Institute 2008. v9.2, USA). Comparison of total mean value of each trait done by used the revised LSD (Petersen, 1985). Pearson correlation coefficient was calculated among studied data.

RESULTS AND DISCUSSION

The data illustrated in figure 1 revealed that concentration of the phenolic compound in fermented soybean was 41.17 mg/100 g with standard deviation 0.12, fermentation of soybean and corn mixture was 37.33 mg/100 g with standard deviation 6.80 while fermentation of corn was 20.10 mg/100 g. The results showed that fermented soybean or corn alone generates much more phenolic content than the mixture. On the other hand, the antioxidant activity of soybean exhibited 8.32 mg/100 g, fermentation of mixture was 7.75 mg/100 g, while fermentation of corn was 7.12 mg/100 g, and the results showed that the fermentation of soybean or corn separately was much better than fermentation together. Total phenolic content increased in soybean after 72 h fermentation, it also activates antioxidants in comparison with the non-fermented soybean (Gracia, *et al.*, 2004). Our results are coinciding with data obtained by Robbins, 2003, who stated that total phenolic content increased in fermented soybean.

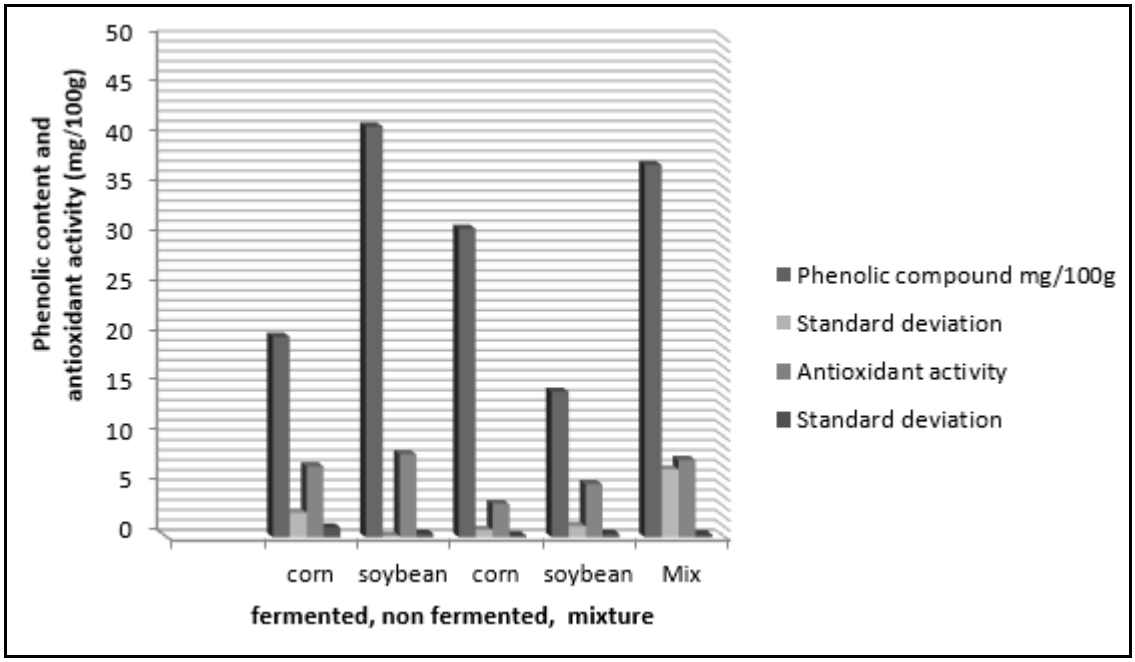

Fig. 1 Phenolic content and antioxidant activity of unfermented and fermented soybean and corn.

Other results showed that fermented soybean was superior to soybean in most antioxidant properties (Yang et *al.*, 2000). The fermentation play an important role in the improvement of antioxidation (Ga and Bae, 2011). Total phenolic and flavonoids contents in soybean supporting potential health benefit of animal feed . In addition, soybean fermentation could enhance the amount of these components (Samruan et *al.*, 2014). It was observed that phenolic compound decreasing during natural lactic acid fermentation of maize (Awada *et al.*, 2005). The increase in total phenolic content found in the fermented samples could be due to the bound phenolic being liberated during fermentation (Bartolome and Gomez-Cordoves, 1999). The decrease in phenolic compounds during fermentation could also be due to the acidic environment and the reduced extractability of the phenolic compounds (Towo, *et al.*, 2006).

Table of 2 shows that the antimicrobial activity of soybean, corn and mix fermentation by *Bacillus subtilis*. Antimicrobial activity of fermented soybean and corn against eight selected microorganisms exhibited high activity by fermented soybean and corn-soybean mixture while fermented corn was fewer antimicrobial activities. Antimicrobial activities of both fermented soybean and soybean-corn mixture against all tested microorganisms, showed that zones of inhibition ranged from 13 to 22 mm. fermented corn exhibited inhibition against *Pseudomonas aeruginosa, Saccharomyces cerevisiae, Candida albicans,* and *Fusarium lateritium* while no inhibition against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Bacillus cereus* by fermented corn.

Antimicrobial properties of fermented mixture (soybean and corn) exhibited. Highest activity with inhibition zone 22 mm for *Fusarium lateritium, Candida albicans,* and 20mm inhibition zone against *Saccharomyces cerviaceae, Pseudomonas aeruginosa* and *Bacillus cereus*, 18 mm inhibition zone against *Staphylococcus aureus, Klebsiella pneumoniae* and 16ml inhibition zone against *Proteus vulgaris*. Antimicrobial activity of soybean showed that 22mm inhibition zone against *Fusarium lateritium,* 20mm inhibition zone against both *Candida albicans* and *Bacillus cereus* 19mm inhibition zone against *Saccharomyces cerviaceae*,17mm inhibition zone against *Staphylococcus aureus*, 15mm

inhibition zone against *Proteus vulgaris*, 14mm inhibition zone against *Pseudomonas aeruginosa*, 13mm inhibition zone against *Klebsiella pneumonia*, while antimicrobial activity of corn was 17mm inhibition zone against *Pseudomonas auroginosa*,12mm inhibition zone against *Candida albicans*, *Fusarium lateritium*, 11mm inhibition zone against *Staphylococcus cerviaceae*. It has been reported that increased antimicrobial activity by fermentation of soybean (Fuller, 1989).

Table 2. Zone of inhibition (zone diameter- mm) of fermented corn and soybean extracts against pathogenic microorganisms.

Microorganisms	Zone of inhibition (mm)		
	CSF	SSF	CSSF
Staphylococcus aureus	0	17	18
Klebsiella pneumoniae	0	13	18
Proteus vulgaris	0	15	16
Bacillus cereus	0	20	20
Pseudomonas aeruginosa	17	14	20
Saccharomyces cerevisiae	11	19	20
Candida albicans	12	20	22
Fusarium lateritium	12	22	22

CSF: Corn Solid-State Fermentation, SSF: Soybean Solid-State Fermentation, CSSF: Corn-Soybean mix Solid-State Fermentation

Figure 2. Shows that soybean total protein increased after fermentation (53.34) comparing with non-fermentable soybean (48.81), also, total protein of fermented corn was increased (13.07) comparing with non-fermentable corn (11.82), whereas total protein of fermented soy-corn mixture was (38.61). This result indicated that the fermentation of each substrate either soybean or corn separately better than fermentation within the mixture. Other studies have shown that soy antigenic proteins could be degraded during fermentation (Chi and Cho, 2016; Frias, 2008). Rozan *et al.*, 1996 showed that at the first stage of fermentation with *B.subtilis* effect on the characteristics of proteins in corn-soybean meal mixed feed.

Our result showed that the amount of protein and ash % after fermentation of corn and soybean increased by 1.88, 4.53, and 1.57, 0.96 respectively (figure 2), whereas carbohydrates and oil decreased by 3.2, 4.51 and 0.73, 0.99 respectively. Other research results showed that there is significant increase of protein during fermentation of maize (43.5%), and total phenolic content (23.4%) (Cui *et al.*, 2012). Fermentation of soybean by *Bacillus natto*, increase of protein content with the highest content in the moisture. On the other hand contents of carbohydrates increased and ranged from 14.77 to 29.08 while those of crude fiber, fat and ash were generally low (Ojokoh and Wei, 2011). Decreasing of carbohydrate and oil contents may be due to that *Bacillus subtilis* utilized carbohydrate and oil during fermentation as the major source of energy (Yamabe *et al.*, 2007).

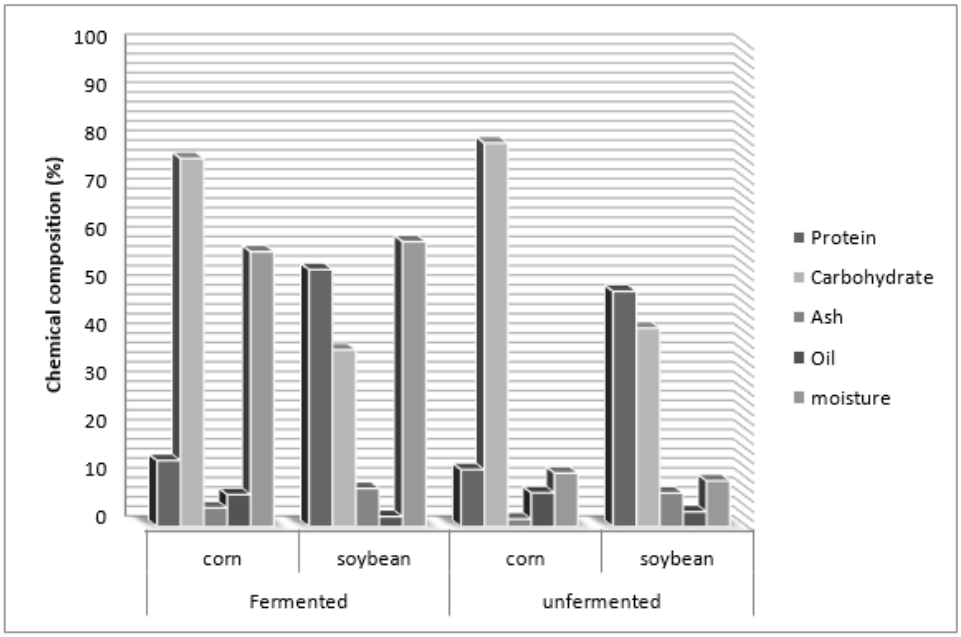

Fig.2: Effect of fermentation on chemical composition of corn and soybean.

Enhancement of antimicrobial and antioxidant activities of substrate either soybean or corn by fermentation may be releasing of bioactive peptides (Singh *et al.*, 2015) or due to generating of bioactive peptides during soybean fermentation (Kwon *et al.*, 2002). In addition to many oligopeptides produced from soy protein by microbial proteinase during fermentation, demonstrated a range of biological activities (Gibbs *et al.*, 2004).

CONCLUSION

From our results, we can conclude that fermentation of both soybean and corn produce significant increase of protein content, antioxidant activity, and antimicrobial activities but negative influence on carbohydrate and oil contents. Therefore, this study recommended that fermented soybeans could be adding to animal feed.

REFERENCES

A.O.A.C. (2000): Official Methods of Analysis of the Association of Official Analytical Chemists International. 17 th ed. Maryland, USA.

Ashok S., Nawa R. and Vedaste N. (2010): Bacillus Fermentation of Soybean: A Review. J. Food Sci. Technol. Nepal. 6, 1-9.

Awada, S.H., Hady, A., Hassan, A. B., Ali, M. I. and Elfadil B., E. (2005): Antinutritional factors content and availability of protein, starch and mineral of maize (Zeamays linnanus) and lentil (Lens culinaris) as influenced by domestic processing. Journal of Food Technology, 3,523–528.

Bartolome, B. and Gomez-Cordoves, C. (1999): Barley spent grain: release of hydroxycinnamic acids (ferulic and p-coumaric acids) by commercial enzyme preparations. Journal of the Science of Food and Agriculture, 79 (3), 435–439.

Bergey, D. H., Krieg, N. R., and Holt, J. G. (1984): *Bergey's manual of systematic bacteriology*. Baltimore, MD: Williams & Wilkins.

Chi, C. H. and Cho, S. J. (2016): Improvement of bioactivity of soybean meal by solid state fermentation with *Bacillus*

amyloliquefaciens versus *Lactobacillus* spp. and *Saccharomyces cerevisiae*. LWT-Food Sci Technol.; 68:619–25.

Cui L., Li D.j. and Liu Cq. (2012): Effect of fermentation on the nutritive value of maize. International Journal of Food Science and Technology 47: 755-760.

Fontana, A. R, Antoniolli, A., and Bottini, R. (2013): Grape pomace as a sustainable source of bioactive compounds: extraction, characterization, and biotechnological applications of phenolics. J Agric Food Chem. 61:8987–9003.

Frias, J., Song, Y. S., Martínez-Villaluenga, C., González DME and Vidal-Valverde C. (2008): Immune reactivity and amino acid content of fermented soybean products.JAgric Food Chem.; 56:99–105.

Fuller, R. (1989): Probiotics in man and animals .J Appl Bacteriol. 66:365–378.

Ga, Y. C. and Bae, J. H. (2011). Comparative Evaluation of Fermented and Non-fermented Soybean Extract on Antioxidation and Whitening Toxicol Res.; 27(4): 205–209.

Gibbs, B. F., Zougman, A., Masse, R. and Mulligan, C. (2004): Production and Characterization of Bioactive Peptides from Soy Hydrolysate and Soy-Fermented Food. Food Research International, 37(2):123-131.

Gracia-Alonso, M.; De Pascual-Teresa, S.; Santos-Buelga, C.; Rivas-Gonzalo, J.C. (2004): Evaluation of antioxidant properties of fruits. Food Chemistry, 84, 13–18.

Hong, H. A., Khaneja, R., Tam, N. M., Cazzato, A., Tan, S., Urdaci, M., Brisson, A., Gasbarrini, A., Barne,s I. and Cutting, S. M. (2009): *Bacillus subtilis* isolated from the human gastrointestinal tract. Research in Microbiology. 160 (2): 134–143.

Ignat, I., Volf, I. and Popa, V. I. (2011): A critical review of methods for characterization of polyphenolic compounds in fruits and vegetables. Food Chem. 126:1821–1835.

Jaramillo-flores, M. E., gonzález-cruz, L., Dorantes-Álvarez, L., Gutiérrez-López, G.F. and Hernández-Sánchez, H. (2003): Effect to the thermal treatment on the antioxidant activity and content of carotenoids and phenolic compounds of cactus pear cladodes (Opuntiaficus-indica). Food Sci. Tech. Int. 9(4): 271–278.

Kaur, C. and Kapoor, H. (2001): Review: antioxidants in fruits and vegetables – the millennium's health. International Journal of Food Science and Technology, 36 (7): 703–725.

Kwon, D. Y., Oh, S. W., Lee, J. S., Yang, H. J., Lee, S. H. and Lee, J. H. (2002): Amino Acid Substitution of Hypocholesterolemic Peptide Originated from GlycininHydrolyzate. Food Science and Biotechnology, 11(3): 55-61.

Lee, S. C., Kim, J. H., Jeong, S. M., Kim, D. R., Ha, J. U., Nam, K.C. and Ahn, D.U. (2003): Effect of far-infrared radiation on the antioxidant activity of rice hulls. Journal of Agricultural and Food Chemistry, 51: 4400-4403.

Li, D. F., Nelssen, J. L., Reddy, P. G., Blecha, F., Hancock, J. D. and Allee, G. L., (1990): Transient hypersensitivity to soybean meal in the early-weaned pig. J. AnimSci.; 68:1790–9.

Limón, R.I.; Peñas, E.; Torino, M.I.; Martínez-Villaluenga, C.; Dueñas, M.; Frias, J. (2015): Fermentation enhances the content of bioactive compounds in kidney bean extracts. Food Chemistry 172:343–352.

Masuko T., Minami A. , Iwasaki N., Majima T., Nishimura S., LeeY C.(2005): Carbohydrate analysis by a phenol sulpuric acid method in microplateformat.Anal.Biochem. 339(1):.69-72.

Nakano, M. M. and Zuber, P. (1998): Anaerobic growth of a "strict aerobe" (*Bacillus subtilis*) .Annual Review of Microbiology. 52: 165–190.

Ojokoh, A.O. and Wei, Y. (2011): Effect of Fermentation on Chemical Composition and Nutritional Quality of Extruded and Fermented Soya Products. International Journal of Food Engineering 7, 4-6.

Pandey, A. (2003): "Solid-state fermentation". Biochem. Eng. J. 13 (2–3): 81–84.

Petersen, R. G. (1985): Design and analysis of experiments. Marcel Dekker, Inc., New York, USA.

Raimbault, M. (1980): "Fermentation en milieu solide: croissance de champignons filamenteu xsur substrat amylacé". These ORSTOM (in French): 1–287.

Robbins, R. J. (2003): Phenolic acids in foods: an overview of analytical methodology. Journal of Agricultural and Food Chemistry. 51(10):2866-2887.

Rozan, P., Villaume, C., Bau, H. M., Schwertz, A., Nicolas, J. P. and Mejean, L. (1996): Detoxication of rapeseed meal by *Rhizopus oligosporus* sp-T3: A first step towards Rapeseed protein concentrate. Int J Food Sci Technol.; 31:85–90.

Samruan, W., Gasaluck, P. , Oonsivilai, R. (2014): Total phenolic and flavonoid contents of soybean fermentation by Bacillus subtilis SB-MYP-1.Advanced Materials Research, 931-932 (2): 1587-1591

SAS Institute. (2008): The SAS system for Windows, release 9.2.SAS Institute, Cary, N.C. USA.

Schillinger, U. (1989): Antimicrobial activity of L. sake isolated from meat. Appl. Environ Microbiol 55: 1901-1906.

Singh BP, Vij S, Hati S, Singh D, Kumari P, Minj J (2015): Antimicrobial activity of bioactive peptides derived from fermentation of soy milk by Lactobacillus plantarum C2 against common foodborne pathogens. Int J Fermented Foods 4:99–99.

Singhania, R. R., Patel, A. K.; Soccol, C.R. and Pandey, A. (2009): "Recent Advances in solid-state fermentation". Biochem. Eng. J. 44 (1): 13–18.

Song, Y. S., Pérez, V. G, Pettigrew, J. E., Martinez-Villaluenga, C. and de Mejia, E. G. (2010): Fermentation of soybean meal and its inclusion in diets for newly weaned pigs reduced diarrhea and measures of immune reactivity in the plasma. Anim Feed Sci Technol.; 159:41–49.

Sudhagar, S. and Reddy R. (2017): Influence of elevation in structuring the gut bacterial communities of Apiscerana Fab. Journal of Entomology and Zoology Studies, 5(3): 434-440.

Towo, E., Matuschek, E. and Svanberg, U. (2006): Fermentation and enzyme treatment of tannin sorghum gruels: effects on phenolic compounds, phytate and in vitro accessible iron. Food Chemistry, 94 (3): 369–376.

Yamabe, S., Kobayashi-Hattori, K., Kaneko, K., Endo, K. and Takita, T. (2007): Effect of soybean varieties on the content and composition of isoflavone in rice-koji miso. Food Chemistry, 100 (1): 369-374.

Yang J.H., Mau J.L., Ko P.T., Huang L.C. (2000). Antioxidant activity and active compounds of fermented soybean broth. Food Chemistry, 71, (2): 249-254.

Zielijski, H., Michalska, A., Ceglijska, A. and Lamparski, G. (2008). Antioxidant properties and sensory quality of traditional rye bread as affected by the incorporation of flour with different extraction rates in the formulation. European Food Research and Technology, 226 (4): 671-680.