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E-mail: [bjisir07@gmail.com](mailto:bjisir07@gmail.com)

## Nutritional and microbiological quality of germinated soy flour

M. M. Rahman, M. M. Rahman\*, U. F. Shahjadee, A. Z. Rupa and M. N. Hossain

*Institute of Food Science & Technology, Bangladesh Council of Scientific & Industrial Research  
Dhaka-1205, Bangladesh*

### Abstract

This investigation was carried out to evaluate the nutritional and microbiological quality of germinated soy flour. Protein content of germinated soy flour was 6.90% higher than the non-germinated soy flour. Essential amino acids content, including Lysine, Valine and Threonine was increased by 8.53%, 8.50% and 7.93% respectively. In addition, vitamin B<sub>2</sub> and iron contents were also increased by 14.29% & 8.80% respectively, compared to non-germinated soy flour. Germination resulted in nutritionally enriched soy flour as compared to non-germinated one. The microbiological quality parameters including total bacterial count, total coliform count, fecal indicator *Escherichia coli* and yeast & mold count was found to be within acceptable level throughout the 6 months of storage at ambient temperature. No food borne pathogen including *Salmonella* spp. *Staphylococcus aureus* was detected. Furthermore, spore forming bacteria including *Bacillus cereus* was not detected in any of the germinated soy flour sample tested. In addition, fungal metabolites including aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> could not be detected in any sample. Nutritional and microbiological finding of this study, indicate the germinated soy flour could be used as raw material or ingredient for making diverse food and bakery products.

**Keywords:** Germinated soy flour; Nutritional quality; Aflatoxins and microbiological quality

### Introduction

Soybean is one of the most important oil and protein crops of the world containing 30 to 45% protein with a good source of all indispensable amino acids (Islam *et al.*, 2007; Serrem *et al.*, 2011). Recently, soybean is extensively and effortlessly grown for making diverse processed foods (Ogunola *et al.*, 2006) and worldwide production was recorded as 217.60 MT in 2005-07 and predicted to increase 2.20% or 371.3MT by 2030 (Tadayoshi and Peter, 2009). However, annual production of soybean in Bangladesh was 1000MT in 2015 and limited use of soybean in making food products except oil production due to the health benefit information gap among the producer and consumers. Since soybean products play an important role in health (Messina and Barnes, 1991; Messina, 1995; Sirtori *et al.*, 1995) and thus have promoted numerous food products derived from soybean such as soybean flour, textured soybean, soybean dairy-like products, meat, bakery products in different countries (Ladodo and Borovik, 1992). Its use in the production of bread as composite flour has been reported (Kure *et al.*, 1998; Dhingra and Jood, 2004; Basman *et al.*, 2003).

Germination is widely claimed as a means of correcting nutrient deficiencies of particular seeds, and soybean sprouts are the perfect example, because all the life-giving proteins, carbohydrates, oils, vitamins and minerals necessary to sup

port life are stored within the seeds. Germination is a complex metabolic process during which the lipids, carbohydrates, and storage proteins within the seed are broken down in order to obtain the energy and amino acids necessary for the plant's development (Jachmanian *et al.*, 1995). Non germinated soybean grains contain some anti-nutritional and toxic compound which inhibit enzyme (Egounlety and Aworh, 2003; Ahia, 2003) and germination could help to overcome these disadvantages (Zhu *et al.*, 2005). Germination process (Freed and Ryan, 1978) and heat treatment (Leontowicz *et al.* 1998) reduced trypsin inhibitor in sprouted soybean (Zhu *et al.*, 2005).

Through this study, efforts have been made to promote and aware consumers with the use of soybean in preparing different food products including protein enriched bread that contains composite flours of soybean and other locally grown crops. Therefore, the objectives of this study were to determine the effects of germination on nutritional and microbiological quality of soybean flour compared to raw soy flour.

### Materials and methods

#### *Collection of Soybean seed*

Soybean seeds were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. For experi-

\*Corresponding author. e-mail: [mahbubar63@gmail.com](mailto:mahbubar63@gmail.com)

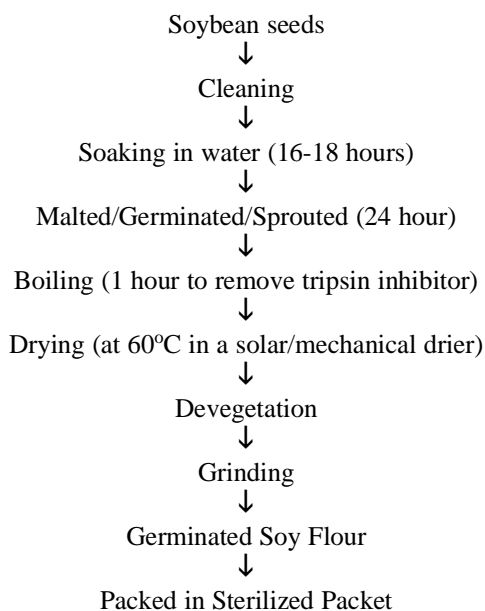
mental analysis, 20 seeds of each kind were placed on water soaked filter paper in sterilized Petri dish. Distilled water was added daily to the experimental seeds. Completely Randomized Design (CRD) was followed for placing the Petri dishes on the laboratory bench. The proximate analysis, amino acids content, minerals and enzyme activities were evaluated of raw and germinated soy flour.

#### *Preparation of germinated Soy Flour*

##### *Processing of soy bean seeds*

The soybean seeds were soaked in 0.5% aqueous calcium hypochlorite solution for 2.0 min and then washed 7-8 times with distilled water to remove hypochlorite residues from the seed surfaces. After surface sterilization, the soybean seeds were soaked in distilled water in a sterilized bowl for 16-18 hours. Then 1.0 kg seeds were placed on sterilized wet cotton cloth in a sterilized tray (4ft x 2ft) in dark condition for germination at room temperature. During the germination, 100ml distilled water was sprayed on soybean seeds to support the germination process and the room temperature was recorded as 19°C. After germination the grains were boiled for one hour to remove tripsin inhibitor and then dried up to 10% moisture content using solar/mechanical drier. The dried soybean were devegetated and ground to make germinated soy flour.

The flow sheet of germinated soy flower is given below:



##### *Proximate analysis*

The proximate analysis including protein, fat, carbohydrate, ash, fiber, moisture and mineral contents of raw and germinated soy flour was evaluated. Protein content was determined using Macro-Kjeldahl method. (Ranganna,1979). The carbohydrate fat, ash, fibre, moisture, and mineral contents (calcium, phosphorus and iron) of soybeans were determined by the method as described in "A Manual of Laboratory Techniques" (Anonymous, 1976).

##### *Enzyme activity assay*

Raw and germinated soy flour (0.5g) was separately grinded in a mortar with cold 0.1M phosphate buffer with respective pH (amylase & invertase at pH 6.7 and protease at pH 5.5) and finally crushed into paste using a homogenizer. The temperature was maintained at 4°C by putting ice in the outer chamber of the homogenizer. The suspension was then filtered through few layers of cheese cloth in cold room. The filtrate was collected and clarified further by centrifugation in a refrigerated centrifuge at 10,000 rpm for 15 min at 4°C.

##### *Amylase*

Amylase activity was assayed by the method as described (Jayaraman, 1981). Starch solution (1.0%) was used as substrate (1.0g in 100 ml of 0.1M phosphate buffer, pH 6.7). The amylase activity was measured by estimating the release of maltose calculated from the standard curve prepared with maltose. One unit of amylase activity was defined as the amount required for liberating 1.0 mg of maltose in 15 min at 37°C.

##### *Invertase*

Invertase activity was assayed by the method as described (Mahadevan and Sridhar, 1982) using sucrose as substrate. The invertase activity was measured by estimating the release of glucose calculated from the standard curve prepared with glucose. One unit of invertase activity was defined as the amount required for liberating 1.0 mg of glucose in 15 min at 37°C.

##### *Protease*

Protease activity was assayed by the method as described (Mahadevan and Sridhar, 1982) using milk protein casein as substrate. The protease activity was measured by estimating

the release of leucine calculated from the standard curve prepared with leucine. One unit of protease activity was defined as the amount required for liberating 1.0 mg of Leucine in 30 min at 37°C.

#### *Amino acids analysis*

Amino acid composition of germinated and raw soy flour was determined by using an amino acid analyzer (Model No: 228-39015-38; Shimadzu, Japan), able to determine fourteen amino acids (Anonymous, 1993). Sample (0.5g) soy flour was pasted with 50ml 6N HCl by mortar pestle, filter and filtrate was hydrolyzed for 22-24 hours in a hydrolyzing apparatus. After hydrolyzing HCl was removed from filtrate with distill water for 3-4 times by evaporation in a water bath. After completing the evaporation, the stock solution was prepared and mark up to 25ml in a volumetric flask by using 0.1N HCl. This stock solution was used for the determination of amino acids.

#### *Microbiological analysis*

##### *Total viable count*

Test sample and microbial media were prepared according to standard operating procedure. The sample suspension and decimal dilutions were prepared following international guidelines (ISO 6887). The test sample 1.0 gram were mixed thoroughly in plate count agar and poured into the petri dishes and kept in the biosafety cabinet at room temperature to solidify. The inoculated petri dishes were incubated upright position in the incubator at 30 °C ± 1 °C for 72 h ± 3 h. All the analysis was done in duplicate and the results was expressed in CFU/g according to the ISO 7218:1996 methods.

##### *Coliforms and E. coli*

Test sample, initial suspension and sufficient number of dilutions were made following the standard method (ISO 6887). The diluted and non-diluted samples were poured in screw cap tube containing double and single strength Lauryl sulfate tryptose broth, EC broth and Brilliant green lactose bile broth separately and incubated at 30 °C or 37 °C for 24 h or 48 h. Three tubes of double and single strength liquid selective enrichment medium are inoculated with a specified

quantity of the test sample if the initial product is liquid, or with a specified quantity of an initial suspension. The most probable number of coliforms per millilitre or per gram of sample (i.e. the MPN) is calculated from the number of tubes in the new series showing gas formation (ISO 4831:2006). A table for determination of most probable numbers is used (ISO 7218, ISO 7251:2005).

##### *Yeast and molds*

The Dichloran Rose Bengal Chloramphenicol (DRBC) and Dichloran 18% Glycerol (DG18) agar media were prepared according to instruction. Serial dilutions of the sample were prepared using peptone solution (ISO 6887). The petri dishes were prepared and inoculated using spread plate method. For high water activity foods ( $a_w > 0.95$ ), Dichloran Rose Bengal Chloramphenicol (DRBC) agar was used and for reduced water activity foods ( $a_w < 0.95$ ), Dichloran 18% Glycerol (DG18) agar was used. The DRBC plates were incubated upright position at 25 ± 1°C for 5 days and DG18 agar plates were incubated for 7 days. Confirmation of individual presumptive yeast colonies was done by microscopic examination, as some bacteria are capable of growth on DRBC agar (ISO 21527-1, 2: 2008). The result was expressed according to international methods (ISO 7218:1996).

##### *Salmonella*

Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Salmonella spp. (ISO 6579:2002, MOD). Pre-enrichment in non-selective liquid medium, Enrichment in selective liquid media, selective agar media, biochemical media and serological reagents were prepared following the international method (ISO 6579:2002, MOD). Test sample diluted in peptone water was inoculated in pre-enrichment in non-selective liquid medium and incubated at 37 °C ± 1 °C for 18 h ± 2 h. Enrichment in selective liquid media Rappaport-Vassiliadis medium with soy (RVS broth) and Muller-Kauffmann tetrathionate/novobiocin broth (MKTT broth) are inoculated with the culture incubated at appropriate temperature for 24 h ± 3 h. Then from enrichment medium the culture were plated onto XLD agar plate for the isolation of lactose-positive Salmonella and Salmonella typhi and Salmonella paratyphi strains.

### *Bacillus cereus*

Initial suspension, decimal dilutions and culture media were prepared according to instruction (ISO 6887) and 0.1 ml of diluted and non-diluted samples were surface plated onto two agar plates and kept the plates for 15 min at room temperature and then incubated the plates for 18 h to 24 h in an incubator at 30 °C. Presumptive colonies from each plate were selected and confirmed by Haemolysis test on sheep blood agar and Biochemical reaction (ISO 7932:2004).

### *Staphylococcus aureus*

Test samples and all the media were prepared according to standard operating procedure. Initial suspension and dilutions were prepared following international guide lines (ISO 6887). The test samples were inoculated in a selective culture medium in screw cap test tubes. The tubes are incubated at 37 °C, anaerobically, for 24 h and 48 h. The presence of presumptive coagulase-positive staphylococci was observed by the reduction of potassium tellurite. The presumptive positive suspension was spread on to Baird-Parker agar plates and incubated at 37 °C for 24 h and 48 h. Typical and/or atypical colonies are confirmed by a coagulase and rabbit plasma fibrinogen reaction (ISO 6888-3:2003).

### *Aflatoxin*

Aflatoxin of germinated soy flour was determined by using HPLC detection with Fluorescence detector (Huq *et al.*, 1999).

### *Statistical analysis*

Each experiment was replicated three times. Reported data represented in the tables are the mean values  $\pm$  SD obtained from three individual experiments. Data were subjected to analysis of variance using the Microsoft Excel program (Redmond, Washington DC, USA.).

### **Results and discussion**

Soybean is an excellent and inexpensive source of high quality protein comparable to meat, poultry and eggs. Germination is a very effective technology for improving the quality of nutritive value of soy flour. Proximate analyses of raw soy flour are described in Table I. The results showed

that protein content of germinated soy flour and raw soy flour was recorded as 41.96% and 39.25%, respectively (Table I). The protein content was increased 6.90% in germinated soy flour. This finding was similar to the other study done by Ugwuona *et al.*, (2012) and Nwosu *et al.*, (2014). The moisture content of germinated soy flour was recorded as 8.90%, while the moisture content in raw soy flour was 8.60% (Table I). The moisture content data obtained in this study were comparable with data reported by Victoria and Felix, (2009). On the other hand, neither increase nor decrease of carbohydrate, fat, fiber, and ash content was observed in germinated and raw soy flour. Vitamin and mineral contents were found higher in germinated soy flour compared to raw soy flour. Germinated soy flour contains 77.06 $\mu$ g/100gm vitamin A, 0.29 $\mu$ g/100gm vitamin B<sub>1</sub>, 0.32  $\mu$ g/100gm Vitamin B<sub>2</sub> whereas raw soy flour contains 74.68 $\mu$ g/100gm vitamin A, 0.26 $\mu$ g/100gm vitamin B<sub>1</sub>, 0.28 $\mu$ g/100gm Vitamin B<sub>2</sub> (Table II). Vitamin B<sub>2</sub> increased maximum 14.29% followed by vitamin B<sub>1</sub>(11.54%) and vitamin A (3.19%). Raw soy flour contains 365mg/100gm calcium, 488mg/100gm phosphorus and 15.12mg/100gm iron. On the other hand, germinated soy flour contains 368mg/100gm calcium, 496mg/100gm phosphorus and 16.45mg/100gm iron (Table II). Highest 8.80% increased of iron content was observed in germinated soy flour whereas phosphorus and calcium contents did not increase as like as iron content in germinated soy flour. These results are in agreement with the literature of Kordyles, (1990) who also reported vitamin and minerals contents were found higher in

**Table I. Nutritional value of raw and germinated soy flour**

Sl No.	Test Parameters	Results	
		Raw Soy Flour	Germinated Soy Flour
1.	Protein (%)	39.25 $\pm$ 0.22	41.96 $\pm$ 1.40
2.	Fat (%)	23.78 $\pm$ 0.85	23.51 $\pm$ 0.65
3.	Ash (%)	3.94 $\pm$ 1.18	4.00 $\pm$ 0.64
4.	Crude Fibre (%)	5.86 $\pm$ 0.71	5.58 $\pm$ 0.65
5.	Carbohydrate (%)	18.57 $\pm$ 1.32	16.71 $\pm$ 0.57
6.	Moisture (%)	8.60 $\pm$ 0.42	8.90 $\pm$ 0.76
7.	Calorie (%)	458.20 $\pm$ 2.82	456.48 $\pm$ 1.66

The values are mean  $\pm$ SD of determinations made in triplicate.

**Table II. Vitamins and minerals of raw and germinated soy flour**

Sl No.	Test Parameters	Results	
		Raw Soy Flour	Germinated Soy Flour
1.	Vitamin A( $\mu\text{g}/100\text{gm}$ )	74.68 $\pm$ 0.34	77.06 $\pm$ 1.29
2.	Vitamin B <sub>1</sub> ( $\mu\text{g}/100\text{gm}$ )	0.26 $\pm$ 0.03	0.29 $\pm$ 0.02
3.	Vitamin B <sub>2</sub> ( $\mu\text{g}/100\text{gm}$ )	0.28 $\pm$ 0.04	0.32 $\pm$ 0.04
4.	Calcium (mg/100g)	365 $\pm$ 3.00	368 $\pm$ 3.00
5.	Phosphorus (mg/100g)	488 $\pm$ 7.00	496 $\pm$ 8.00
6.	Iron (mg/100g)	15.12 $\pm$ 1.2	16.45 $\pm$ 0.40

The values are mean  $\pm$ SD of determinations made in triplicates

malted soya flour than raw soya flour during 24 hour germination.

Amino acids content in raw and germinated soy flour were presented in Table III. Essential amino acids content including Threonine (3.13%), Lysine (2.29%), Valine (0.51%), Glutamic acid (1.28%), Isoleucine (1.49%) and Methionine (1.28%) were found higher in germinated soy flour than raw soy flour. Among the essential amino acids analyzed, maximum 8.53% increased of Lysine was observed in germinated soy flour followed by Valine (8.50%), Threonine (7.93%), and Methionine (7.5%). Furthermore, among the non-essen-

**Table III. Amino acid content of raw and germinated soy flour**

Sl No.	Parameters	Results	
		Raw Soy Flour	Germinated Soy Flour
1.	Aspartic acid	2.11	2.25
2.	Threonine	2.90	3.13
3.	Serine	1.12	1.23
4.	Glutamic acid	5.22	5.57
5.	Glycine	1.48	1.61
6.	Alanine	0.98	1.09
7.	Valine	0.47	0.51
8.	Methionine	1.19	1.28
9.	Isoleucine	1.38	1.49
10.	Leucine	0.59	0.63
11.	Tyrosine	1.44	1.56
12.	Histidine	0.40	0.43
13.	Lysine	2.11	2.29
14.	Arginine	1.75	1.89

tial amino acid analyzed, Alanine content increased maximum 11.22% followed by Serine (9.82%), Glycine (8.78%), Tyrosine (8.33%) and Arginine (8.00%) in germinated soy flour compared to raw soy flour. Hallen, 2004 reported that amino acids and available vitamins were increased during germination and similar finding was obtained in this study.

Microbiological quality of six months stored germinated soy flour and the presence of naturally occurring aflatoxin were presented in Table IV. The initial total mesophilic aerobic bacterial population was recorded as  $2.6 \times 10^2$  CFU/g and slightly increased to  $3.8 \times 10^3$  CFU/g after six months of storage at room temperature. The initial total coliforms bacteria was recorded as non-detectable level and increased to 35 MPN/g after six months of storage at room temperature (Table IV). Fecal indicator bacteria *Escherichia coli* were not observed throughout the storage period. Yeast & mold count was found within acceptable level throughout the 6 months of storage at ambient temperature and no food borne pathogen including *Salmonella* spp. *Staphylococcus aureus* was detected. Furthermore, spore forming bacteria including *Bacillus cereus* was not detected in any of the germinated soy flour sample tested. In addition, fungal metabolites including aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> were not detected in any sample (Table IV), these results are in accordance with Dipika & Krishna, (2010). Final product was stored six months for microbiological observation by maintaining the moisture content below 9.0%. North Dakota State University reported that flour containing higher than 14.0% moisture is not microbiologically stable at room temperature. Moisture content of germinated and non-germinated soy flour in this

**Table IV. Microbiological quality and Aflatoxin assessment of germinated soy flour stored at room temperature for six months**

Sl No.	Test Parameters	Results		
		0 Days	3 Months	6 Months
1.	Mesophilic aerobic bacteria, cfu/g	$2.6 \times 10^2$	$2.4 \times 10^2$	$3.8 \times 10^3$
2.	Total Coliforms, MPN/g	<3*	21	35
3.	<i>E. coli</i> , MPN/g	<3*	<3*	<3*
4.	Total yeasts and molds, cfu/g	<10**	<10**	<10**
5.	<i>Salmonella</i> sp./25g	Not detected	Not detected	Not detected
6.	<i>Staphylococcus</i> sp. cfu/g	<10**	<10**	<10**
7.	<i>Bacillus cereus</i> /25g	Not detected	Not detected	Not detected
8.	Aflatoxins (B <sub>1</sub> ,B <sub>2</sub> , G <sub>1</sub> ,G <sub>2</sub> )	Not detected	Not detected	Not detected

\*Most probable number <0.3 indicates absence of test organisms in 1.0 g sample.

\*\*<10 indicate absence of test organisms in 1 g of sample.

study was in supportive range for safe storage as describe by Ihekoronye and Ngoddy, (1985).

### Conclusion

The study results concluded that germinated soy flour is a protein, vitamin & mineral rich food ingredients that could be used to prepare diversified food products with enriched nutritional value. Raw soy flour contains some anti-nutritional factors which can be removed through germination process. Germinated soy flour can be stored up to six months at ambient temperature and was found microbiology safe for human consumption. Therefore, considering the nutritional and microbiological finding of this study, the germinated soy flour could be used as raw material or ingredient for making diverse food and bakery products.

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### References

Ahia AC (2003), The influence of solvent extracted soybean flour in sorghum wort beer. *Nigerian Food J.*, **21**: 94-98.

Anonymous (1976), Manual of Laboratory Techniques. National Institute of Nutrition, Indian Council of Medical Research, Hyderabad-500007; India, 3-5.

Anonymous (1993), Amino acid analysis system instruction manual. Shimadzu HPLC amino acid analysis system. Shimadzu Corporation. Analytical Instruments Division, Kyoto, Japan, 63-65.

Basman A and Koxsel H (2003), Utilization of Transglutaminase use to increase the level of barley and soy flour incorporation in wheat flour breads. *J. Food Sci.*, **68**(8): 2453-2460.

Bau HM, Villaume C, Nicolas JP and Mejan L (1997), Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soybean seeds. *J. Sci. Food Agric.*, **73**:1-9.

Dhingra S and Jood S (2004), Effect of flour blending on functional, baking and organoleptic characteristics of bread. *Int. J. Food Sci. Technol.*, **39**: 213-222.

Dipika AM and Krishna J (2010), Effect of drying on nutritional and functional and electrophoretic pattern of soyflour from sprouted soybean (*Glycine max*), *J. Food Sci. Technol.*, **47**(5): 482-487.

Dugje IY, Omoigui LO, Ekeleme F, Bandyopadhyay R, Lava Kumar P, and Kamara AY (2009), Farmers' Guide to Soybean Production in Northern Nigeria.

Egounlety and Aworh OC (2003), Effect of soaking, dehulling, cooking and fermentation with *Rhizopus*

- oligosporus on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (*Glycine max* Merr.), cowpea (*Vigna unguiculata* L. Walp) and groundbean (*Macrotyloma geocarpa* Harms). *J. Food Eng.*, **56**: 249-254.
- Freed RC and Ryan DS (1978), Changes in the kunitz inhibitor during germination of soyabeans: An immune electrophoresis assay system. *J. Food Sci.*, **43**: 1317-1319.
- Hallén E, Ibanoglu S and Ainsworth P (2004), Effect of fermented/ germinated cowpea flour addition on the rheological and baking properties of wheat flour. *J. Food Eng.*, **63**: 177-184.
- Huq MS, Amin MR, Dawlatana M, Shahida S, Rahim M and Khan SA (1999), Investigation on the occurrence of aflatoxin/mycotoxin in food grains and feed stuffs of Bangladesh: part-1: aflatoxin in rice, *Bangladesh J. Sci. Ind. Res.*, **34**(1): 136-143.
- Ihekoronye AI and Ngoddy PO (1985), Integrated food science and technology for the tropics. London: Macmillian Publisher Ltd.p- 75-76.
- Islam T, Chowdhury A, Islam M and Islam S (2007), Standardization of Bread Preparation from Soy Flour. *Int. J. Sustain. Crop Prod.*, **2**(6): 15-20.
- ISO 4833:2003 Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of microorganisms-Colony counts technique at 30°C.
- ISO 6887 Microbiology of food and animal feeding stuffs-Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.
- ISO 7218:1996, Microbiology of food and animal feeding stuffs-General rules for microbiological examinations.
- ISO 4831:2006, Microbiology of food and animal feeding stuffs-Horizontal method for the detection and enumeration of coliforms-Most probable number technique.
- ISO 7251:2005, Microbiology of food and animal feeding stuffs-Horizontal method for the detection and enumeration of presumptive *Escherichia coli*-Most probable number technique.
- ISO 21527-1, 2:2008 Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of yeasts and molds.
- ISO 6579:2002, MOD Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp.
- ISO 6888:2003 Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species), Part 3: Detection and MPN technique for low numbers.
- ISO 7932:2004 Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of presumptive *Bacillus cereus*-Colony-count technique at 30°C.
- Jachmanian V and Mukherjee KD (1995), Germinating Rapeseed as Biocatalyst: Hydrolysis of Oils Containing Common and Unusual Fatty Acids. *J. Agric. Food Chem.*, **43** (11): 2997-3000.
- Jayaraman J (1981), Laboratory Manual in Bio-chemistry, Wiley Eastern Ltd. New Delhi, India.
- Junwei Z and Kye-chung P (2005), Methyl Salicylate, A Soybean Aphid-Induced Plant Volatile Attractive to the Predator, *J. chem. Ecol.*, **31** (8) : 1733-1746.
- Kordyles JM (1990), Processing and Preservation of Tropical and Subtropical Foods. Machmillan publishers, London. p- 67-80.
- Kure OA, Bahago EJ and Daniel EA (1998), Studies on the proximate composition and effect of flour particle size of acceptability of biscuits produced from blends of soyabeans and plantain flours. *Namoda Technol. Scope J.*, **3**(2): 17-22.
- Leontowicz H, Kostyra H, Leontowicz M and Kulasek GW (1998), The inactivation of legume seed haemagglutinin and trypsin inhibitor boiling. In: Jansman AJM,

- Hill GD, Poel V (eds) Recent advances of research in antinutritional factors in legume seeds and rape seeds. The Netherlands, Wageningen, p- 429-432.
- Mahadevan A and Sridhar R (1982), Methods of Physiological plant pathology (2<sup>nd</sup> Ed.) Sivakasi publication, Madras, India. p- 316.
- Messina M and Barnes S (1991), The role of soy products in reducing risk of cancer. *J. Natl. Cancer Inst.*, **83**: 541-546.
- Messina M (1995), Modern uses for an ancient bean: soy foods and disease. *Chem. Znd.*, **11**: 412-415.
- Nwosu JN, Owuamanam CI, Omeire GC and Eke CC (2014), Quality parameters of bread produced from substitution of wheat flour with cassava flour using soybean as an improver, *American J. Res. Commu.*, **2** (3): 99-118.
- Olguin MC, Hisano N, D' Oltavio AE, Zingale MI, Revelant GC and Caderari SA (2003), Nutritional and antinutritional aspects of an Argentinean So Flour assessed on weaning rats. *J. Food Compo. Ana.*, **16**: 441-449.
- Otunola ET, Ogunsola O and Abioye VF (2006), Effect of temp on some properties of 'Agidi', A West African fermented maize gel. *Int. J. Food Agric. Res.*, **3**(1):119-128.
- Ranganna S (1991), Handbook of analysis and quality control for fruit and vegetable products. 2<sup>nd</sup> Ed. (Tata McGraw-Hill Publisher, New Delhi), pp- 12-27.
- Serrem C, Kock H, Taylor J (2011), Nutritional quality, sensory quality and consumer acceptability of sorghum and bread wheat biscuits fortified with defatted soy flour. *Int. J. Food Sci. Technol.*, **46**: 74-83.
- Sirtori CR, Lovati MR, Manzoni C, Monetti M, Pazzucconi F and Gatti E (1995), Soy and cholesterol reduction: clinical experience. *J. Nutr.*, **125** (3 Suppl.): 598S-605S.
- Tadayoshi M and Goldsmith PD (2009), World soybean production: Area harvested, yield and long term projections. International Food and Agribusiness Management Review volume 12. Issue 4.
- Ugwuona FU, Ogara JI and Awogbenja MD (2012), Chemical and sensory Quality of cakes formulated with wheat, soybean and cassava flours, *Indian J.L. Sci.*, **1**(2):1-6.
- Zhu D, Hettiarachchy N, Horax R, Chen P (2005), Isoflavone contents in germinated soybean seeds. *Plant Foods Hum. Nutr.*, **60**: 147-151.

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