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Full Length Research Paper

Effect of fermentation on nutritional and anti-nutritional properties of fermenting Soy beans and the antagonistic effect of the fermenting organism on selected pathogens

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ABSTRACTS

Soybeans seed were bought from market cleaned pulverized and processed in to fermentation stages for analysis on physiochemical, proximate, organoleptics and microbial load parameters. This studies reveals the effect of Physiochemical changes that occurred on fermentation of soybeans per period to range from; moisture (19-65)% sugar (2.28-2.07)% pH (6.8 - 9.5), Temperature (18 - 28)^{0}C respectively, Proximate composition; crude protein (48.17 - 58.05), crude fibre (17.20 - 20.16), fat content (13.48-18.44)% ash (3.04 - 3.62)% TTA (1.10 - 1.45)% Organoleptic changes; colour (Butter-light brown), texture (fine-sticky), smell (pleasant- chocking), Microbial load also varies on different selective media: Nutrient agar for total count (1.2 x 10^3-2.8 x 10^3), Mannitol salt agar for *Staphylococcal* count (1.6 x 10^1-1.0 x 10^1), tryptose soy agar for *Bacillus* count (1.5 x 10^1 - 8.2 x 10^1), PDA for fungi count (3.0 x 10^3-3.3 x 10^3), MacConkey for coliform count (Nill). The effect of the *Bacillus* spp was very pronounce against *Staphylococcus aureus* at 0.8 cm zone of inhibition and was not effective against *Candida albican.* It can therefore be concluded that *Bacillus* spp are the major fermenting organism of soybeans and its effect against the selected pathogen could implies it as a good probiotic microbes.

Keywords: Organoleptic, Proximate, Pathogens, Antinutrients, Antagonistic.

INTRODUCTION

Traditional food systems are considered to be the backbone of modern food industries. Fermented soya bean foods are common in the orient and other part of the world. In Nigeria, condiment is another important fermented soya beans product. *Glycine soja* is the wild ancestor of *Glycine max,* and grows wild in China, Japan Korea, Taiwan and Russia. The subgenus Glycine consists of at least 16 wild perennial specie for examples, *Glycine canescens.*

Soya beans (*Glycine max)* ranks high among the vegetable protein sources. The crop is particularly value for it high protein content. Recently in Nigeria a stimulated interest appears to have developed in the use of Soya beans for human food. One of such steps is commercial production of soy-ogi, a protein fortified Nigeria made beverages, which was derived, perfected and produced at the federal institute of industrial research Oshodi Nigeria.

The fermented product compares favourably with 'Iru' another condiment prepared from locust bean (*Parkia filicoidae)* seeds. Soybean (*Glycine max* L) is known to have a characteristic objectionable fishy or paint-like odour and anti nutritional factors like trypsin inhibitor. Consumption of raw soybean causes growth depression, pancreatic hypertrophy, hyperplasia and adenoma in experimental animals (Rackis and Gumbmann, 1981). On the other hand, fermented soybean has a wide range of health benefits like anticancer effects (Yee, 2000), reduction of menopausal syndrome frequency, osteoporosis protection (Anderson, 2000; Yee, 2000) and coronary heart disease prevention due to anti-atherogenic properties.

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There are a lot of microorganisms present in the fermentation of soya beans for the condiment production. A variety of bacteria grows in fermenting soya beans during fermentation and products mucilaginous substances that cover and links the individual beans cotyledons. This research work seek to achieve the following aims

1 To carry-out the effect of fermentation on the physiochemical properties of fermenting soybeans seeds per fermentation period.
2 To carry out the effect of fermentation on the proximate composition of the fermenting soybeans seed per fermentation period.
3 To carry out the effect of fermentation on the organoleptic properties of fermenting soybeans seed per fermentation period.
4 To isolate and characterize the resident microbes on soy beans flour/ powder.
5 To carry out the effect of fermentation on the microbial load of the fermenting soy beans seed per fermentation period.
6 To carry out the antimicrobial effect of the fermented soybeans on clinically selected pathogens.
7 To carry out the antimicrobial effect of the fermenting microbial isolate from fermenting soybeans on clinically selected pathogens.

MATERIALS AND METHODS

Source of samples

The samples, soybeans were purchased at Oja Oba Owo, in Owo Local Government of Ondo state, Nigeria.

Fermentation of the samples

The fermentation procedure was carried out under aseptic condition according to the method of (**Denis, 1980**). One kilogram of the samples were cleaned, washed with cleaned water and boiled in 500ml of tap water for 30mins. The beans were allowed to cool for one hour and the seed coats were removed (dehulling). The beans were them sieved and boiled with another 500ml of tap water for 2hours and transfer into a clean cool water of 1000ml beaker. Aluminum foil was used to cover the surface of the beaker firmly the beaker containing beans was incubated at 37^0C for 5 days.

Proximate analysis of fermented soya beans

The proximate analysis fat, crude protein, crude fiber, free fatty acid, Temperature changes, pH, moisture content and Minerals, were carried out according to the method of AOAC (1990).

Antinutrient components

Antinutrient Tannin (Total Phenol), Phytate, Alkaloid, Oxalate, Flavonoids of fermenting soybeans was quantified per fermentation day in g/100g according to AOAC (1990).

Preparation of culture media

The culture media used were Nutrient agar (NA), a general purpose media used for total mesophillic bacteria count, Tryptose soybean agar for *Bacillus* count, Mannitol salt agar for *Staphylococcal* count, PDA for Fungal count, and MacConkey agar for Coliform count. The culture media were prepared according to manufactures instructions and then sterilized in the autoclave at 121^0C for 15minutes.

Microbial isolation

The samples were serially diluted, 10^{-4} ml of the diluted sample was pipetted aseptically into appropriately labeled sterile petridishes. The media was poured aseptically into the different labeled petridishes. The plates were swirled gently in a planar circular motion to ensure uniform distribution and uniform growth of the organisms on the agar. The media was allowed to solidify. Plates were incubated at 37^0C. The various bacterial colonies isolated were sub-cultured to obtained pure cultures. They were later identified based on their colonial morphology, biochemical characteristics and cellular morphology. The 24 hours old culture was sub-cultured to have pure isolate by the use of streak plating method and also incubated for 24 hours. After 24hours 2 (two) isolate was obtained from the sample.

Total Viable count

The number of colony forming per unit of the bacteria was done, to know the quantitative analysis of the microbe.

Antimicrobial activities

Antimicrobial activities were tested using agar well diffusion method according to the method of Akinyemi *et al.* (2005). Two ml of identified *Bacillus* spp, isolated as the major bacteria responsible for the fermentation of the soybeans was prepared in nutrient broth and introduced into the well dug in the agar plate against five clinically selected pathogenic viz. *Staphylococcus aureus, Candida albican, Proteus, Escherichia coli and Pseudomonas aeruginosa* from Federal Medical Center

Owo. The plates were swirled gently in a planar circular motion to ensure uniform distribution and growth of the organisms on the agar, after solidification, a cork bore was flame sterilized and used to bore holes on the agar, 2ml each of the samples was then put inside the holes, antibiotic was used as positive control, while water serves as negative control the plates were incubated at 37^0C or 24hrs.

RESULT AND DISCUSSION

The physicochemical changes that occurred as a result of fermentation of soybeans for 5 days ranges from % moisture content, % sugar content, pH value and temperature ^{0}C; are present in table 1. There is a significant difference at probability level $p<0.05$ at the rate that physicochemical parameters changes per day of fermentation using analysis of variance as the F calculated (17.01) is greater that the F critical (3.49) of the information in the table 1.

The % moisture content decreased steadily per day ranging from 65% to 19% for 5 days. This could be as result of hydrolytic action of the fermenting microbes. Also, the soybeans are soaked and cooked prior to fermentation had high moisture content. With subsequent fermentation and storage, the moisture content reduced to 60% on the 2nd day of fermentation period. This is because the fermentation occurs at a high temperature and could lead to reduce humidity. Also the soybean solute rises as the solvent decreases. Similar result was also observed according to Thingom and Chhety (2011). The temperature also increases and this could be associated with the thermal emission during fermentation, this is in line with the work of Chaney (1979).

The pH value of the raw soybeans seeds (6.8) is a close value pH value observes in the work of Thingom and Chhety (2011) who work on the nutritional analysis of fermented soybeans with the value of the boiled soybeans seeds to be (6.68). The pH of fermented soybean increased with fermentation time, pH 6.8 to pH 9.5 respectively for 4 days (Table 1). Increasing pH during fermentation has been attributed to proteolytic activities and the release of ammonia following the utilization of amino acids by microorganisms involved in the fermentation released ammonia is mainly responsible for the pungent smell that usually accompanies most vegetative protein fermentation (Sarkar *et al*., 1993).

The content of total soluble sugar of raw soybean seed decreases ranging from (2.7%) from the first day of fermentation till (2.28%). Soaking and cooking resulted in partial loss of oligosaccharides for *natto* production (Kanno *et al*., 1982). The reducing sugar level decreased during the processing of soybean for *Hawaijar* production but increased slightly during fermentation. The increased level of reducing sugar is a reflection of the activities of a-amylase and sucrose in the fermenting seeds (Omafuvbe *et al.,* 2000). The sugar level also decreases per day; this could be as result of its utilization by the metabolizing microbes involved in the fermentation processes. The result these physicochemical changes during fermentation are in line with the work of Giwa *et al*. (2011), who also observed decreases in the sugar content and increases in pH and temperature by fermentation period.

The proximate composition of the product reveals that protein increased due to fermentation, whereas fat contents decreased most likely due to their utilization by the growing microorganisms (Table 2). There is a great significant difference at probability level $p<0.05$ at the rate that proximate profile changes per day of fermentation and also the rate at which each parameter responds at a particular day using ANOVA.

(Dike and Odunfa, 2001) also, reported, high protease activity, which, in turn increased the amino acid content of the product. The increase in amino acid content with fermentation time is especially important from the nutritional point of view as it would increase digestibility and absorption.

The crude fibre decreases from 20.16 to 17.20 per five days respectively. This could be as result of the degradation of the fibre by fermenting microbes. The decreases in fat content could also be attributed to the metabolizing organism as energy source. The trends of changes in the crude protein, fat and fibre are in inline with the work of Ganiyu (2006) who work on nutrient and antinutrient composition of condiments produced from fermented legumes. Ash content did not show much change during the processing of soybean for formation. The fermentation effect on the ash content varied per day, the highest valued recorded was on the third day of fermentation at 3.77% but later decreases to 3.04 on the last day of fermentation.

Table 3 below shows the physical changes of soybeans seeds during fermentation for 5 days. The changes observes are organoleptic proprieties which ranges from colours; butter to light brown, texture; fine to stick, smell; pleasant, irritating to chocking" per day respectively. These changes could be associated with fermentation which induced alteration in food thereby the protein fat and nucleic acids may also be degraded with consequent effect upon food flavour and texture Komolafe (2002). The preparations of food by fermentation process are dependent upon the production by certain microorganism of chemical and physical changes that altered the appearance, body and flavour of the original material. These changes may improve the nutrition of the product and they are generally inhibitive to the growth of undesirable microorganism.

The antinutrient factor determined in table 4, include Tannin, Phytate, Alkaloid, Oxalate and Flavonoid. There is a significant difference at probability level $p<0.05$ at the

Table 1. Physicochemical changes that occurred during fermentation of soybean seeds

Days	Moisture %	Sugar %	pH	Temperature^{0}C
O	64±1.41	2.60±0.14	6.65±0.21	17.5±0.71
1	61.5±2.12	2.58±0.11	7.0±0.00	20.5±0.71
2	52.5±3.54	2.50±0.00	7.55±0.07	24±1.41
3	32±1.41	2.20±0.14	7.9±0.14	26.5±0.71
4	20±1.41	2.14±2.20	9.0±0.71	28.5±0.71

Table 2. Proximate composition of the fermented soybeans

Day's	Crude protein	Crude fiber	Fat content	Ash	TTA %
0	48.19±0.02	20.08±0.11	18.22 ±0.31	3.31±0.44	1.05±0.07
1	50.15±0.21	18.96±0.06	17.27±0.28	3.25±0.36	1.06±0.08
2	50.30±0.04	18.32±0.17	15.05±0.07	3.38±0.54	1.07±0.09
3	52.70±0.99	17.15±0.21	14.10±0.14	3.37±0.52	1.13±0.18
4	56.53±2.16	17.05±0.21	13.24±0.34	3.02±0.03	1.23±0.32

Table 3. Physical changes of soybean seeds during fermentation

Days	Colour	Texture	Smell
0	Butter colours	Fine	Pleasant
1	Butter colours	Sticky	Irritating
2	Butter colours	Sticky	Choking Irritating
3	Light brown	Sticky	Choking
4	Light brown	Sticky	Choking

Table 4. Antinutrient profile of fermenting soybeans per day measured in g/100g

Days	Tannin	Phytate	Alkaloid	Oxalate	Flavonoids
0	1.25±0.35	17.40±0.57	8.02±0.03	1.05±0.07	6.12±0.17
1	1.15±0.21	15.35±0.49	7.74±0.34	1.04±0.05	5.06±0.08
2	0.69±0.92	13.25±0.35	7.32±0.45	1.03±0.04	2.18±0.25
3	0.65±0.49	13.05±0.07	7.15±0.21	1.02±0.28	2.06±0.78
4	0.63±0.53	12.25±0.35	7.10±0.14	1.02±0.21	1.60±0.06

rate that antinutrient parameter changes per day of fermentation and also the rate at which each parameter responds at a particular day using ANOVA.

The presence of these antinutritional factors is one of the major factors limiting the nutritional quality of soybean (Kakade *et al.,* 1969). Phytate was recorded as the highest in value of 17.8 and Oxalate 1.10 g/100g before fermentation. It is evident that phytic acid has a major role in influencing the digestibility of vitamins and minerals. Phytic acid in its natural form as a phytate-minerals-protein complex decreases the availability of zinc, manganese, copper, molybdenum, calcium, magnesium and iron. There was a general noticeable reduction in the entire antinutrient quantified for per day of fermentation. This could be attributed to the activities of the indigenous microbes as well processing that which could initiate the activities of some indigenous enzyme that degrade these antinutrients (Mubarak, 2005).

The total number of the bacterial load increase per day of the research fermenting period which ranges from 1.2×10^3 cfu /ml, 2.4×10^3 cfu / ml, 2.5×10^3 cfu / ml, 2.6×10^3 cfu / ml and 2.8×10^3 cfu / ml per fermenting period respectively (Table 5). The increase in the microbial load could be associated to the fact that the microbes are in there stationary phase. Thereby the available nutrients encourages there growth. These loads

Table 5. Total mesophillic viable Mean Total, Staphylococcus, Bacillus Coliform counts and Total fungal count *(cfu*/g) of fermenting soybeans per day on Selective Media

Day	TVC	TSC	TBC	TCC	TFC
0	1.2×10^3	1.6×10^1	1.5×10^1	Nil	3.0×10^3
1	2.4×10^3	1.5×10^1	3.0×10^1	Nil	3.2×10^3
2	2.5×10^3	1.2×10^1	5.3×10^1	Nil	3.2×10^3
3	2.6×10^3	1.1×10^1	7.1×10^1	Nil	3.3×10^3
4	2.8×10^3	1.0×10^1	8.2×10^1	Nil	3.3×10^3

*Mean values for duplicate samples, TVC-Total Viable Count, NA-Nutrient Agar, TSC-Total Staphylococcal Count, MSA-Mannitol Salt Agar, TBC-Total Bacillus Count, TSA-Tryptose Soy Agar, TCC-Total Coliform Count, TFC- Total Fungi Count and PDA-Potatoes Dextrose Agar

Table 6. Antagonistic effect of *Bacillus* isolate from fermented Soybean against some clinically selected pathogen as zone of inhibition in cm

Names of organism	Zone of inhibition (mm)
Staphylococcus aureus	8.0
Candida albican	-
Proteus	2.0
E. coli	4.0
Pseudomonas aeruginosa	3.0

could be as a result of both normal flora in the fermenting soybeans and contaminants from the environment and during production. The bacterial evaluation results of the fermenting soy beans on different fermenting agar showed a mean Staphylococcal counts of 1.0×10^1 -1.6×10^1, Bacillus counts of 1.2×10^1 -8.2×10^1 and Coliform counts of zero respectively (table 5). The principal isolates were identified as *Staphylococcus sp and Bacillus sp. Staphylococcal* count reduces by fermentation period, while *Bacillus* count increases per fermentation period. The high value of the *Bacillus* count is expected as it has been reported as the major normal flora in foods stuff, agricultural product and soil (Aworh, 1985). The presence of *Bacillus spp* isolated could be attributed to contamination of raw materials from the farm. This reminds us of microbiological purchasing specification that of ICNSF (1974), that raw materials have to met set standard. *Bacillus species* is a popular thermophillic (hay bacillus) that could be brought with raw materials into the factory. The slightest industrial fault could permit them into the final stages of production since they are thermo stable. The official microbiology standard of foods requires the complete absence of coliform and that organism such as *Staphylococcus aureus, Bacillus species* and other spore forming, revivable microorganism should not be more than 10^2 cell/ml, (WHO, 1985). The inhibition activity by the *Bacillus* isolate from fermented soybean against various selected isolates observed was stated on the Table 6. These suggest that this fermented soybean prevents invasion by potential pathogens. As observed in table 3, *Staphylococcal* count was reducing per the fermentation period; these could also be associated with high range in the zone of inhibition (0.8 cm) that occurs in Table 6. *Candida* spp was not inhibited; this implies that the fermenting soybean could be a rich media for the isolate and that the *Bacillus* could not exhibit antagonistic effect on the organisms. These could also imply that *Candida* yeast is one of the major fermenting organisms. The inhibition against *Escherichia coli*, intestinal pathogens could also imply that the use of fermenting soybeans could exert a probiotic effect in the enteric region. *Bacillus* species constituted about 90% of microorganisms found in fermentation of soybeans while Lactic acid bacteria constituted almost 22% (Odunfa, 1981). The exertion of the antagonistic effect of fermenting soybeans on pathogens therefore could be the synergistic effect of both organisms and mechanism of action could be as a result of production of bacteriocin. Cambell-platt (1980) also reported that *Penicillium spp*, fermenting oval budding yeast and film yeast *Candida* were incidental contaminate the majority of bacterial found in Iru are aerobic after 36 hours of fermentation. Odunfa (1981) first reported that the predominant fermentation microorganism was a *Bacillus subtilis* and other species and he also confirmed the present of *Bacillus pumilis, Bacillus licheniformis* in the fermentation.

CONCLUSION

It can be concluded that Fermentation period produce significant and noticeable effect on physiochemical parameters, proximate composition, organoleptic properties, antinutrient composition and microbial load of fermenting soybeans. The microbial isolate, *Bacillus* spp, as the major observable fermenting microbe that possess antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus, Proteus* and *Pseudomonas aeruginosa* is not effective against *Candida spp,* and with the result of research work observed it is therefore recommended that well fermented soybeans powder could be taking by infants, in malnutrition or as supplement because of the high nutritional value and its probiotics effect.

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