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Original Research Article

Analysis of the shelf life of soya bean (*glycine max*) flour

Abstract

This work was to investigate the shelf life of freshly and stored soybean flour by isolating resident bacteria and carrying out their proximate analyses. The bacteria were isolated and identified using standard morphological and biochemical tests. The antibiotic susceptibility of the isolated bacteria was also carried out using standard methods. Isolated bacteria belonged to genera Staphylococcus, Bacillus, Escherichia and Enterobacter. The number of isolated organisms was higher in all cases in the stored flour sample. The results showed that the percentage of crude protein (37.02%), crude fat (16.41%) and dry matter (91.07%), was highest in the freshly purchased soybean flour. The moisture content of the stored flour (9.09%), while carbohydrate (35.21%), ash (3.92%) and crude fibre (4.23%) were also higher. Resistance to antibiotics was highest to cloxacillin (100%), amoxicillin (75%) and augmentin (75%). The result of this work showed that long term storage is detrimental to the soybean flour and the presences of antibiotic resistant bacteria have serious public health implications.

Key words: Shelf life, Soybean, Microbial load, Proximate analysis.

Introduction

The Soybean (*Glycine max*), which is considered to be a miracle bean by many people, was first grown as a crop in China about 5000 years ago [Liu *et al .,* 2008] and has been widely consumed as folk medicines in China, India, Japan and Korea for hundreds of years [Jeng *et al .,* 2010]. Today it is a major source of plant protein (70%) and oil (30%) and has become a globally important crop [Chan *et a*l., 2012].

It is widely used as edible bean. It has numerous uses and the most commonly used food product in the orients are bean curd, various forms of fermented paste bean flour, soybean sprout, numerous health drink and oil (International Institute of Tropical Agriculture, IITA, 1990). According to their different uses, soybean cultivars are classified as grain- type, which are conventional soybeans for oil and animal feeding, and food type which are those for human consumption in fermented foods (misso, tempeh and natto) and non fermented foods (tofu, soy flour and soy milk) [Liu,1999].Soybean oil is highly consumed world-wide and soy milk is often used as a milk substitute to people who have lactose intolerance [Rosenthal *et al.,* 2003].

The seeds of various varieties of soybeans may be spherical, elongated and flat in nature, but the industrial varieties are particularly yellowish in colour and oval in shape [Iwe and Onuh, 1992].Soybean oil and protein content together account for about 60% of dry soybeans by weight with protein at 40% and oil at 20%. The remainder consists of 35% carbohydrate and about 5% ash. Soybean cultivars comprise approximately 8% seed coat or hull, 90%cotyledons and 2%

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hypocotyls axis or germs. Most soy protein is relatively heat-stable storage protein. This heat stability enables soy food products requiring high temperature cooking, such as tofu, soy milk and textured vegetable protein (soy flour) to be made. Soybean is capable of producing the greatest amount of protein for man. This unique plant is generally regarded as an excellent food crop for the protein deficient countries of the world [Liu, 2000].

In Nigeria, progress has been made especially at International Institute of Tropical Agriculture (IITA) Ibadan, in the development of different varieties of soybean that could readily adapt to our ecological condition [Solabi, 1993]. It was introduced into Nigeria in 1908, production rose from 150,000 metric tonnes in 1988 to 300,000 metric tonnes in 1989with production reaching 440,000 metric tonnes in 2002, as a result of industrial and domestic use [Fatoba *et al*., 2011; Iwe, 2003]. In spite of these achievements, the potentials of soybean, which is not only rich in oil but contains protein of high biological value, have not been adequately exploited in the manufacture of different types of food products in Nigeria.

Antibiotics are commonly used to treat diseases caused by bacteria pathogens. Constant antibiotics consumption has led to a rise in resistance over time. Antibiotic resistant bacteria have been found to be transmitted from food to humans and this has led to life-threatening cases and treatment failures. The essence of this work was to isolate bacterial flora of freshly prepared and stored (6 months) soybean flour; carry out the proximate analysis of the flour as well as checking the sensitivity of the isolates to readily available antibiotics.

Materials and methods

Sources and preparation of the sample

Two samples of soybean flour used for this study were bought from Roundabout market in Iwo, Osun State. After purchase, the flour samples were divided into two; half was packaged in airtight polyethylene bags and kept at room temperature for 6 months while the other half was worked upon immediately. The samples were transported to the Microbiology Laboratory of the Department of Biological Sciences, Bowen University Iwo for further analyses.

Enumeration of Coliforms and total heterotrophic bacteria

Serial dilution of the samples of the soybean flour was carried out. Total viable heterotrophic aerobic counts were determined using the pour plate technique. Molten Plate count agar, Mannitol Salt agar, and Eosin Methylene Blue agar were poured into Petri dishes containing 1 ml of different dilutions. The agar media were used to isolate total heterotrophic bacteria (PCA), *Staphylococcus* sp (MSA) and coliforms (EMB) respectively, incubation was done at 37°C for 24h. Bacteria isolates were characterized and identified using standard morphological(Gram reaction and cell morphology) and biochemical (Oxidase, catalase production, citrate utilization ,indole production, methyl red, Voges Proskauer reaction, starch hydrolysis, and sugar fermentation) tests . The tests were performed according to the methods described by Arora and Arora (2000), Fawole and Oso (1988) and identification was carried out according to Bergey's Manual of Determinative Bacteriology (Holt, 1994).

Proximate Analysis

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Proximate analyses were carried out by determining the moisture, crude, protein, fat and ash content of each sample of the flour. These were carried out in duplicates according to the standard methods of AOAC (2005).

Antibiotic sensitivity testing.

A 0.1 ml overnight actively growing broth culture containing $1x10^6$cfu/ml of each bacterial isolate was introduced into a Petri dish and 20 ml of molten agar added. The antibiotic sensitivity discs (HJ04/P, Abtek Biologicals Ltd.) consisting of different antibiotics namely nitrofurantoin (300 µg), augmentin (30 µg), tetracycline (10 µg), gentamycin (10 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), amoxylin (25 µg), erythromycin (5 µg), cloxacillin(5 µg), cotrimoxazole (25 µg), andofloxacin (30 µg), were placed on the solidified agar surface. The plates were incubated overnight at 37°C. The relative susceptibility of each isolate to each antibiotic was shown by a clear zone of inhibition [Bauer *et al.*, 1966].

Results and Discussion

Bacteria belonging to four genera were isolated. The isolated genera were *Staphylococcus*, *Bacillus*, *Escherichia* and *Enterobacter* as shown in Figure 1. The percentage occurrence was higher in Sample A (stored sample) than in Sample B (fresh sample). It has been observed that the primary causative agents of microbial spoilage of food are the bacteria, followed by yeast and mould [FAO, 1979]. The isolation of these microorganisms from the soybean flour was an indication of their involvement in the contamination and spoilage of the product. It also shows the evidence of proteolytic and lipolytic activity [Turner, 1983; Agwung *et al.,* 2006; Eze, 2008]. It has also been reported that the following genera of bacteria, *Bacillus, Micrococcus, Pseudomonas, Staphylococcus,* and *Enterobacter* are involved in the breakdown of fat, oil and protein [Osuji, 1977; Turner, 1983; Agwung *et al*., 2006]. The presence of *Staphylococcus aureus*, which is a normal flora of the skin, indicates contamination during the processing and storage from the food handlers. The consumer is at risk of food poisoning caused by the ingestion of an enterotoxin produced which is characterized by diarrhea and vomiting [Singleton, 1995; Frazier and Westhoff, 2004]. *Bacillus* sp which is a Gram-positive spore former and a fermenting organism was also isolated. They can be found in soil, water, air, and on vegetation. *Bacillus* sp is also capable of causing food poisoning by the production of enterotoxin [Thomas, 1994; Brooks *et al*., 2005]. *E.coli* which is Gram-negative and an enteric organism is an indication of feacal contamination of the food sample. It causes gastroenteritis especially in infants and young children [Turner, 1983; Brooks *et al*., 2004].

Sample A had the highest number of all bacteria isolates, The percentage occurrence of the organism in sample A (Fig 1), *Staphylococcus aureus* was 40%, 25% was *Bacillus* sp, while 30% and 10% were for *E.coli* and *Enterobacter* respectively. In sample B, 25% of the isolate were *staphylococcus aureus and* the order of occurrence for the remaining organisms were 15%, 20% and 10% respectively.

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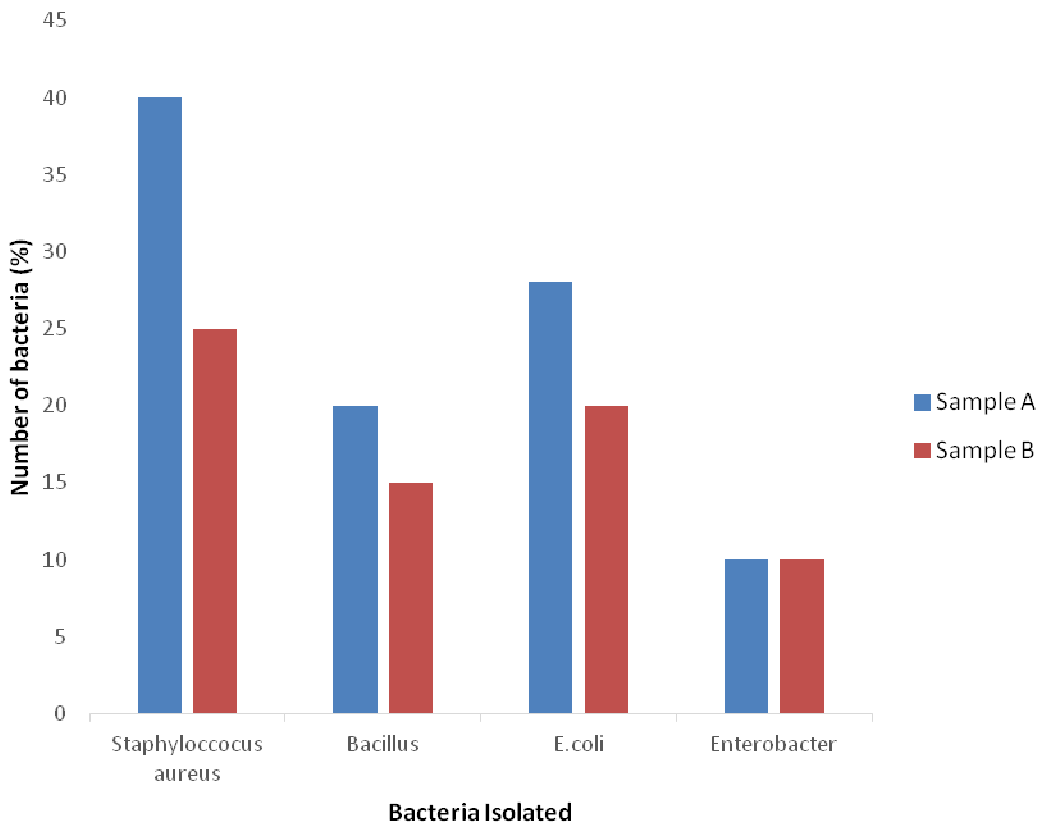

Figure 1: Genera of Bacteria Isolated from the two Flour samples

Sample A: Stored soy bean flour, Sample B: Fresh soy bean flour

The results of the proximate analyses of the two samples are shown in Table 1. Sample B (fresh flour) has a higher protein value (37.02%), protein value in Sample A (33.78%) could have been reduced due to proteolysis during storage which is as observed by [Iwe 2003]. Protein is essential in the synthesis of new cells and body maintenances; this therefore makes it a good food for infants, adults and the elderly. The ash content is an indirect indicator of mineral level in the food product. Ash content in Sample A (3.92%) was higher than that of B (3.76%) which can be attributed to the variety and geographical location of the crop. The ash content in this products falls within acceptable standard of not exceeding 8% (Codex 1989).

The moisture content gives the flour a better shelf life as flour that has low moisture will stay longer and microorganism will not be able to grow on. Both samples had moisture contents of less than 10% as prescribed by Codex 1989. Sample B had a lower value of 8.93%. This may be attributed to the pre- drying and roasting of soybean before milling into flour. Moisture content of sample A was a little higher and this may be due to the absorption of atmospheric moisture during storage, Salunkhe *et al*. [1992] reported that soybean is a good source of unsaturated fat. Fat content for sample A (13.77%) was lower than that of sample B (16.41%) and this may be due to the effect of lipolytic enzymes degrading the fat present during storage.

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Table 1. Proximate composition of soybean flour on dry weight basis

Sample	A	B
Crude protein (%)	33.78	37.02
Crude fat (%)	13.77	16.41
Crude fibre (%)	4.23	3.17
Ash (%)	3.92	3.76
Dry matter (%)	90.09	91.07
Moisture (%)	9.09	8.93
Carbohydrate (%)	35.21	30.68

Values are mean of duplicate analyses

KEY: Sample A: Stored soy bean flour

Sample B: Fresh soy bean flour

Table 2: Antibiotic Sensitivity of Isolated bacteria

	Zones of Inhibition (mm)										
	CO	**CH**	**AU**	**AM**	**ER**	**TE**	**CX**	**GE**	**OF**	**NI**	**NA**
Staphylococcus aureus (A)	**-**	**-**	**-**	**-**	**-**	**14**	**-**	**-**	**NT**	**NT**	**NT**
Bacillus pumilus (A)	**18**	**20**	**-**	**-**	**-**	**-**	**-**	**9**	**NT**	**NT**	**NT**
Bacillus subtilis (A)	**22**	**22**	**-**	**-**	**22**	**-**	**-**	**14**	**NT**	**NT**	**NT**
Staphylococcus aureus (B)	**30**	**34**	**-**	**-**	**-**	**18**	**-**	**26**	**NT**	**NT**	**NT**
Bacillus pumilus(B)	**26**	**14**	**-**	**-**	**-**	**-**	**-**	**20**	**NT**	**NT**	**NT**
Bacillus subtilis (B)	**22**	**22**	**10**	**10**	**34**	**16**	**-**	**22**	**NT**	**NT**	**NT**
E. coli (A)	**-**	**NT**	**18**	**30**	**NT**	**-**	**NT**	**14**	**18**	**16**	**18**
E. coli (B)	**-**	**NT**	**-**	**-**	**NT**	**16**	**NT**	**16**	**18**	**-**	**-**

Key -: no zone; NT: not tested

CO: co-trimoxazole; CH: chloramphenicol; AU:augmentin; AM: amoxicillin; ER: erythromycin; TE: tetracycline; CX: cloxacillin; GE; gentamycin; OF: ofloxacin; NI: nitrofurantoin; NA: nalidixic acid.

Table 2 gives the result of the sensitivity of the isolates to readily available antibiotics. The isolated bacteria were observed to be resistant to at least two antibiotics and this has serious implications to health of those that consume the food if prepared inadequately. Resistance to augmentin and amoxicillin was 75% respectively while resistance was 100% to cloxacillin. From the results, the isolate were resistance to readily prescribed antibiotics, hence the resistance issue could have been caused by overuse or misuse of antibiotics. Multi-drug resistance has been

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recorded in many isolates from food sources [Akponie and Akpan, 2013]. It is encouraged that these foods should be heat treated properly before its consumption.

Conclusion

Soybean is an important and economically valuable food. It is highly nutritious and can be used to solve various malnutrition problems. From the results, it was observed that long storage has detrimental effect on the chemical and nutritional composition of the soybean flour. The bacteria isolated from the samples were also an indication of health hazards to consumers. With the level of resistance, use of unprescribed antibiotics as well as misuse of prescribed ones should be avoided.

The soybean flour is best used fresh as long term storage will lead to the microflora utilizing the components and rendering it unfit for consumption.

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