Nigerian Journal of Tropical Agriculture, vol. 3 2002

PRESERVATION OF SOY FLOUR *(Glycine max. (L.) marr)* USING GINGER (*Zingiber officinale*) AS A NATURAL PRESERVATIVE.

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ABSTRACT

The possibility of preserving soy flour by using different concentrations of ginger extract of 1.0 mg/ 10 g soy flour, 0.5 mg/10 g soy flour and 0.1 mg/10 g soy flour over a period of sixty days was investigated. The experiment revealed that the pH of the treated and untreated samples were in the range of 6.50-6.83 and 6.35-6.65 respectively over the period of sixty days. The bacterial and fungal isolates identified on 60th day of storage from the untreated samples (controls) were Staphylococcus aureus, Bacillus subtiles, Escherichia coli, Aspergillus sp., Penicillium sp. , Mucor sp., Rhizopus sp. and Candida sp. whereas the organisms isolated from the samples treated with 0.5 mg/10 g concentration were found to be Bacillus cereus, Bacillus subtilis , Aspergillus sp. and Rhizopus sp. The total bacterial count for untreated samples was $9.9x10^{10}$ cfu/g whereas the respective value was $7.7x10^{8}$ cfu/g for samples treated with 0.5 mg/10 g concentration. The fungal count for untreated samples was found to be $9.9x10^{10}$ cfu/g whereas the respective count was negligible for samples treated with 0.5 mg/10 g concentration. The protein content (25.1%) and the fat content (18.0%) for untreated samples were much less than those of the treated samples (protein 35% and fat 19.6%) with 0.5 mg/10 g soy flour. The study clearly shows that zinger extract at a low concentration of 0.5 mg/10 g may be used to preserve the soybean flour at least over a period of sixty days.

INTRODUCTION

Protein in the human diet is derived from several sources including cereals, vegetables, root crops, legumes and animal products. Although meat is generally considered the best source of protein due to its complete amino acid composition, the cost of animal protein is rising steadily and most people in developing countries are not able to afford them. Several alternatives to animal protein are being investigated to alleviate protein energy malnutrition problem and soybean is considered one of the best options. Compared with other sources of plant protein, soybean has a superior amino acid profile but it is deficient in methionine and cystine (Okoruwa and Dashiell,1997) . Though there are some anti nutritional components namely trypsin inhibitors, hem agglutinins, urease and phytic acid present in soybean, these can be inactivated by heat especially such as roasting, micro waving and extrusion cooking(Konan and Agbo,1997).

Soy flour has many applications all over the world namely Com-soy milk(CSM), wheat soy blend, soft cheese and soy sauce(Obatolu et al.1993). Soybean lecithin is the main source in the Agro-food Industry for making pastries and sauces (Ducerf, 1990).. Despite all the numerous uses for which soy flour has been recognized, little or no attention has been paid to the study of the nutritional and microbiological changes of soy flour during storage and how it could be preserved to increase the shelf-life.

Because of the variations in microbial content of different types of flour, the type of spoilage in flour is difficult to predict. If acid forming bacteria are present, an acid fermentation begins followed by alcoholic fermentation by yeasts. In the absence of lactic acid bacteria and coliform, micrococci have been found to acidify the flour and in their absence species of *Bacillus* may grow. It is characteristic of most flour paste to develop an odor of acetic acid and esters. Occurrence of pathogenic microorganisms in cereal has been reported. (Aswan, 1983). Among the microbial groups involved in contamination and spoilage of locally produced flours are *Bacillus, Micrococcus, Escherichia* and *Klebsiella.* Presence of spore formers like *Bacillus* is of particular importance because, if present, they could produce toxin (Hesseltine,1968).Moulds develop in succession and cause spoilage of dried foods such as flour if pockets of moisture develop under humid conditions. Okagbue (1986) also reported that yeasts are more hydrophilic than moulds and it is likely that maize flour which usually yield yeasts which has a relatively high moisture content. The major factors for spoilage of cereals by moulds especially the *Asperg* and *Penicillia* include microbial content, moisture level of about 12-13% and physical damage and temperature. Fungi known to damage soybean includes *Diaporthe phaseolorum, Colltotrichum dematium* var. *truncats* and *Cercospora kikuchii* (McDonald, 1985).

Many spices have been reported to possess antimicrobial properties and have been successfully used as preservatives (Shelef,1983). The ethanolic extract of ginger was selected for preservation purpose due to the fact that the aqueous extract produced less microbial activity than the ethanolic ones (De et al, 1999). The objective of this study is to preserve soy flour for a period of sixty days using a natural preservative, *Zngiber officinale*, and to determine the microbiological and nutritional quality of soy flour over sixty days of storage.

MATERIALS AND METHODS

Collection of materials

Five hundred grams of soybean seeds, TGX 536-02D, was collected from the Crop Production Department in the School of Agricultural Engineering, Federal University of Technology, Minna, Nigeria. The underground stem of *Zingiber officinale* was purchased in dried form from Minna main market. The amount of extract for 100 g of ginger was 3.68 g.

Preparation of materials

Soy flour

Washed soybean seeds were oven dried at 55°C for 24 hours and then ground into fine powder using a milling machine as described by Konan and Agbo,1997. After sieving, the powdered soybean was kept in a sterile container for subsequent use.

Extract of ginger

Dried ginger was ground into powder using an electric blender. The 100 g of sample was extracted with 400 ml of water in a 2 liter conical flask for 24

hours. Extract was then recovered by filtration using Whatman no. 1 filter paper. A rotary evaporator was used, in vacuo, at 40°C to concentrate the extract (Irobi and Daramola,1993) . The dried extract was used for preservation purpose.

Phytochemical analysis of Z. officinale

Preliminary phytochemical analysis was carried out using the method described by Fadeyi and Akpan (1989). The spice was screened for alkaloids, anthranoids, anthraquinones, tannins, polyphenols, sesquiterpenes, saponins and tannins. Alkaloids, tannins and polyphenols were found to be present in the extract of *Z. officinale*.

Treatment of soy flour with ginger extract

Thirty milligrams of ginger extract was dissolved in 3 ml of acetone to obtain a stock solution of 10 mg/ml concentration. To 100 g of soy flour 1 ml of this solution was added to get a concentration of 1 mg/10 g soyabean flour and mixed vigorously using a sterile spoon. The treated soybean flour was dispensed in 10 polythene bags each containing 10 g and were sealed with an electric sealer and were kept for 60 days at room temperature(30-32°C) for 60 days. Similarly soy flour was separately treated with ginger extract at 0.5 mg/10 g and 0.1 mg/10 g and were kept for 60 days. Soy flour without any extract was used as control and also kept for 60 days for comparison purpose.Two of the samples for each concentration and control were taken on 7th, 14th, 30th and 60th day for microbiological and biochemical analysis purpose.

Quality assessment of treated and untreated samples

pH determination

One gram of each of treated and untreated samples was added to 10 ml of distilled water and after vigorous shaking the pH was measured using a pH meter (Micro pH 3310 Crison).

Isolation and Enumeration of fungal and bacterial isolates

Each of the samples (0.1 g) was added to 9.9 ml of distilled water and using this as a stock solution, serial dilution upto 10^{-8} were made following the procedure of Fawole and Oso (1988). Zero point one ml of each dilution was introduced onto dried agar medium (for bacterial isolates, nutrient agar, NA, and for fungal isolates, potato dextrose agar, PDA, was used) and the plates were incubated at 28°C and 37°C for PDA and NA plates respectively. The total bacterial and fungal count was expressed in cfu/g. The bacterial isolates were identified using the procedure of Hudson and Sherwood (1997) and fungal isolates were identified using the procedure developed by Smith (1977).

Determination of moisture, fat and crude protein content

The **moisture content(%)** was determined using an electric protimeter grain master 2000 . The **fat content(%)** of the treated and untreated soy flour was determined by direct soxhlet extraction using petroleum ether as solvent. Two grams of soy flour

for each sample was used for this purpose (A.O.A.C, 1980). The total nitrogen content of the samples were determined by Kjeldahl method (Bermner, 1965) and then the **crude protein content** of the soy flour was determined by multiplying the total nitrogen content by a factor of 6.25.Two hundred and fifty milligrams of soy flour was used for this purpose.

The amount of extract for 100 g was 3.68 g. The extract of *Z. officinale* was found to contain the following: alkaloids, tannins and polyphenols. The pH range for both the treated and untreated samples was 6.50-6.95 and 6.40-6.90 for the sixty days of the study. The bacterial and fungal organisms for both treated and untreated samples is stated as follows:

RESULTS

Table 1: Relative distribution of isolates in untreated(UTS) and treated (TS) samples (mean ± SEM; n=3

Isolate	% distribution				
	0 day	60th day			
	UTS	TS			
		UTS	ZO1	ZO2	ZO3
Staphylococcus aurous	18.3± 0.4	16.4± 0.3	-	-	-
Bacillus cereus	12.4 ±0.1	16.5± 0.2	57.3± 0.1	54.3± 0.3	36.3± 0.2
Bacillus subtilis	20.4 ± 0.2	17.7 ± 0.2	40.9± 0.3	41.6± 0.0	22.2± 0.1
Escherichia coli	-	9.9 ± 0.1	-	-	4.3± 0.0
As, ,llus sp.	10 .2± 0.2	20.0 ± 0.1	0.6± 0.2	3.8± 0.1	17.2 ± 0.3
Penicillium sp.	7.3 ± 0.6	8.0 ± 0.5	-	-	4.9± 0.0
Mucor sp.	7.0 ± 0.1	4.0 ± 0.0	-	-	5.6 ± 0.2
Rhizopus sp.	18.4 ± 0.2	2.9 ± 0.2	-	-	6.0± 0.1
Candida sp.	5.3 ± 0.2	3.6 ± 0.2	-	-	3.3± 0.0

ZO!- samples treated with 1mg/10 g concentration of *Z. officinale*

ZO2- " " " 0.5mg/10 g " " "

ZO3- " " " 0.1 mg/10 g " " "

In case of untreated samples on 0 day of storage, *Staphylcoccus aureus, Bacillus cereus* and *Bacillus subtilis, Aspergillus* sp., *Penicillium* sp., *Mucor* sp., *Rhizopus* sp. and *Candida* sp. were isolated from NA plates and PDA plates whereas *Escherichia coli* was isolated in case of untreated samples on 60th day of storage. For treated samples at 1.0 mg/10 g concentration and 0.5 mg/10 g concentration *Bacillus cereus, Bacillus subtilis* and *Aspergillus* sp. were isolated on 60th day of storage. For treated samples at 0.1 mg/ 10 g concentration *B. cereus, B. subtilis, E. coli , Aspergillus* sp., *Penicillium* sp., *Mucor* sp., *Rhizopus* sp. and *Candida* sp. were isolated. The results are shown in Table 1 above.

Table 2: Total number of bacterial isolates in untreated(UTS) and treated samples(TS)

(mean± SEM where n=3)

Time Interval (day)	UTS	TS		
		ZO1	ZO2	ZO3
0	$1.1x10^8 \pm 0.3$	$1.1x10^8 \pm 0.2$	$1.1x10^8 \pm 0.3$	$1.1x10^8 \pm 0.3$
7	$1.4x10^{10} \pm 0.3$	$8.8x10^8 \pm 0.3$	$9.0x10^8 \pm 0.3$	$9.9x10^8 \pm 0.3$
14	$9.0x10^{10} \pm 0.3$	$7.5x10^8 \pm 0.3$	$8.0x10^8 \pm 0.1$	$9.3x10^8 \pm 0.4$
30	$9.0x10^{10} \pm 0.2$	$6.9x10^8 \pm 0.4$	$7.9x10^8 \pm 0.1$	$7.5x10^8 \pm 0.1$
60	$9.9x10^{10} \pm 0.2$	$6.5x10^8 \pm 0.4$	$7.7x10^8 \pm 0.2$	$7.6x10^8 \pm 0.4$

ZO1- samples treated with 1mg/10 g concentration of *Z. officinale*
ZO2- " " " 0.5mg/10 g " " "
ZO3- " " " 0.1 mg/10 g " " "

For control samples, the initial bacterial count was $1.1x10^{10}$ and on 60th day of storage the count was $9.9x10^{11}$. For samples treated with different

concentrations of extracts of ginger, the range of count was $6.5x10^8$ –$7.7x10^8$ on 60^{th} day of storage. The results are shown in Table 2.

Table 3: Total number of fungal isolates in untreated (UTS) and treated (TS) samples(mean± SEM; n=3)

Time Interval (day)	UTS	TS		
		ZO1	ZO2	ZO3
0	$1.9x10^8$±0.2	$1.9x10^8$± 0.3	$1.9x10^8$± 0.1	$1.9x10^8$± 0.3
7	$3.1x10^{10}$±0.3	$7.0x10^8$± 0.1	$3.3x10^8$± 0.1	$1.1x10^6$± 0.3
14	$4.3x10^{10}$±0.4	$2.0x10^6$± 0.2	$1.4x10^7$± 0.2	$2.0x10^7$± 0.1
30	$5.1x10^{10}$ ±0.2	NG	NG	$1.0x10^6$± 0.3
60	$9.9x10^{10}$ ±0.1	NG	NG	$1.0x10^6$± 0.1

ZO1- samples treated with 1mg/10 g concentration of *Z. officinale*
ZO2- " " " 0.5mg/10 g " " "
ZO3- " " " 0.1 mg/10 g " " "
NG- negligible

For ginger extract treated samples at 1.0 mg/10 g and 0.5 mg/10 g the fungal count on 60^{th} day was negligible whereas the count was $1.0x10^6$ in case of treated samples at 0.1 mg/10 g concentration. The results are shown in Table 3 above.

The **moisture content** of powdered soy flour on 0 day of storage was 17.6%. The moisture content of treated and untreated samples of soy flour on 7^{th}, 14^{th}, 30^{th} and 60^{th} day of storage are listed in Table 4.

Table 4: Determination of moisture content(%) of untreated (UTS) and treated (UTS) samples(mean± SEM; n=3).

Sample	time interval (days)				
	0	7	14	30	60
UTS	16.9± 0.1	16.0 ± 0.1	16.1 ± 0.0	16.1± 0.1	16.2 ± 0.2
TS(ZO1)	"	17.5 ± 0.2	15.3 ± 0.1	15.2 ± 0.2	15.2 ± 0.2
TS(ZO2)	"	17.1 ± 0.3	14.8± 0.1	14.8 ± 0.3	14.8 ± 0.3
(ZO3)	"	14.9 ± 0.1	14.4± 0.1	13.2 ± 0.2	13.2 ± 0.1

ZO1- samples treated with 1 mg/10 g concentration of *Z. officinal*
ZO2- " " " 0.5mg/10 g " " "
ZO3- " " " 0.1 mg/10 g " " "

The reduction in **fat content** for untreated samples over 60 days of storage was 2% whereas for treated samples at 1.0 mg/10g, 0.5 mg/10 g and 0.1 mg/10 g the respective values were 0.6%, 0.8% and 1.0%. The reduction in **protein content** for untreated samples was 15% whereas the respective values for treated samples were 7%, 4.5% and 5.7%. The results are shown in Table 5 below.

Table 5: Determination of fat and crude protein content of treated(TS) and untreated samples(UTS) (mean;± SEM; n=3).

Time Interval (day)	UTS		TS					
			ZO1		ZO2		ZO3	
	F	P	F	P	F	P	F	P
0	20.0±0.1	40.0±0.2	20.0±0.2	40.0±0.0	20.0±0.0	40.0±0.3	20.0±0.2	40.0±0.1
7	19.4±0.2	35.0±0.1	19.5±0.1	37.1±0.1	19.3±0.2	37.2±0.1	19.4±0.1	36.1±0.0
14	19.0±0.2	33.8±0.2	19.2±0.2	35.7±0.2	19.4±0.1	36.9±0.2	19.2± 0.2	36.0±0.2
30	18.6±0.1	28.6±0.0	19.4±0.1	33.4±0.0	19.2±0.0	35.6±0.1	19.0 ± 0.0	35.0±0.1
60	18.0±0.0	25.1±0.2	19.2±0.2	33.1±0.2	19.6±0.3	35.5±0.2	19.0± 0.2	34.3±0.2

ZO1- samples treated with 1 mg/10 g concentration of *Z. officinale*
ZO2- " " " 0.5mg/10 g " " "
ZO3- " " " 0.1 mg/10 g " " "
F and P represent % fat and % of crude protein in the samples.

Discussion

Whole soybean seeds were selected considering the fact that the hull contains 8% of soy protein and 6% fibre. Whole soy flour prepared in this study contains 40% crude protein and 20% fat. This agrees with the crude protein (42%) and fat content (20%) of the whole soy flour reported by Bressani(1981). The study detected the presence of three bacterial species namely *S. aureus*, *B. cereus* and *B. subtilis* in soy flour samples on 0 day of storage. The fungi isolated on 0 day of storage were species of *Aspergillus; Penicillium, Mucor, Rhizopus* and *Candida*. Though drying, cleaning and washing of grains and the milling of flour reduce the number of microorganisms, some organisms still in whole grain flour may be due to the handling problems and the spoilage would be similar to that of cereal grains (Frazier and Westhoff, 1994). The initial pH of the soy flour samples was 6.90 and over 60

Table 4: Determination of moisture content(%) of untreated (UTS) and treated (UTS) samples(mean± SEM; n=3).

Sample	time interval (days)				
	0	7	14	30	60
UTS	16.9± 0.1	16.0 ± 0.1	16.1 ± 0.0	16.1± 0.1	16.2 ± 0.2
TS(ZO1)	"	17.5 ± 0.2	15.3 ± 0.1	15.2 ± 0.2	15.2 ± 0.2
TS(ZO2)	"	17.1 ± 0.3	14.8± 0.1	14.8 ± 0.3	14.8 ± 0.3
(ZO3)	"	14.9 ± 0.1	14.4± 0.1	13.2 ± 0.2	13.2 ± 0.1

ZO1- samples treated with 1 mg/10 g concentration of *Z. officinal*
ZO2- " " " 0.5mg/10 g " " "
ZO3- " " " 0.1 mg/10 g " " "

The reduction in **fat content** for untreated samples over 60 days of storage was 2% whereas for treated samples at 1.0 mg/10g, 0.5 mg/10 g and 0.1 mg/10 g the respective values were 0.6%, 0.8% and 1.0%. The reduction in **protein content** for untreated samples was 15% whereas the respective values for treated samples were 7%, 4.5% and 5.7%. The results are shown in Table 5 below.

period could be due to the production of acidic metabolites by these organisms during growth. The initial bacterial count was $1.1x10^{10}$ whereas the count was $9.9x10^{11}$ on 60th day of storage for control samples. The bacterial count was in the order of 10^8 for all the treated samples. In this study, ginger was seen to inhibit the growth of *E. coli* and *S. aureus* but not *B. cereus* and *B. subtilis*. De et al.(1999) also reported that ethanolic extract of ginger inhibits the growth of *E. coli* to a great extent. The initial fungal count was $1.9x10^{10}$ and on 60th day of storage the count was $9.9x10^{10}$. Some workers claim that 15% or above moisture permits good fungal growth and therefore the small amount of moisture present in soy flour (17.6%) brings about the proliferation of fungal growth. The presence of *B. cereus* in stored soy flour though at very low level is a cause of concern because this might cause food poisoning resulting from *B.cereus* which can withstand heat, this result agrees with that of Blakey and Priest (1980). Aflatoxin is not considered a problem in soy bean storage though Farag et al.(1986) detected aflatoxin in sterilized and non-sterilized soybeans inoculated with *A. paraciticus*. For ginger treated samples at 1.0 mg/10 g and 0.5 mg/10 g concentrations there was no fungal growth on 30th day and 60th day of storage but for samples treated with 0.1 mg/10 g the count was $1.9x10^6$. Doty (1961) reported that maximum counts specified for the flour used in various food $5.0x10^3$- $1.5x10^4$. Ginger is reported to inhibit a number of fungal species namely *Aspergillus, Fusarium* and *Candida*. De et al. (1999) reported that the ethanolic extracts of *Z. officinale* possess significant antimicrobial activities against *S. avium, C. prfringens, E. coli, A. niger* and *A. flavus*. The reduction in crude protein (%) for the control over 60 days was significantly high compare to the samples treated could be due to the reduced microbiological activities in the treated samples due to reduced bacterial and fungal load.

So, it may be concluded that soy flour, a protein supplement to cereal grains, prepared by using this roasting technique is of high nutritional quality. Ginger at 0.5 mg/10 g concentration may be one of the most effective preservative for soy flour over 60 days of storage. Though the reduction in protein content is minimal for zinger treated samples at 0.5 mg/10 g concentration the bacterial count on 60th day of storage was $6.5x10^8$ which is high compared to the values recommended for stored flour and it contained some pathogenic organisms such as *B.cereus* which can withstand heat.

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